

**Epidemiology and burden of soil-transmitted helminth infections
among school-aged Bulang children
in Yunnan province, People's Republic of China**

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Dekan

*Dedicated to my parents
and husband*

“Thoroughly conscious ignorance is the prelude to every real advance in science.”

James Clerk Maxwell (1831-1879)

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2. Summary

Background The three most common soil-transmitted helminths are *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Ancylostoma duodenale* and *Necator americanus* (hookworms). Collectively, they infect around 1 billion people and put approximately 5 billion people at risk of infection worldwide. *Strongyloides stercoralis* is a less common soil-transmitted helminth than the species mentioned above, but still significant from a public health perspective. The global prevalence of *S. stercoralis* is estimated to be 30-100 million. Populations most affected by these parasitic worms are often impoverished, living on less than US\$ 2 per day, and have poor sanitation facilities and hygiene habits. Despite the low prevalence levels of soil-transmitted helminth infections observed on a national level in the People's Republic of China (P.R. China), hotspots of these infections, particularly within ethnic minority groups residing in rural areas, still exist.

Single-species infections with the common soil-transmitted helminths can cause symptoms ranging from abdominal pain to anaemia, and impaired development of cognitive abilities. For *S. stercoralis* infections, symptoms such as severe abdominal pain and blood in the stool have been reported. Due to the chronic and subtle nature of most morbidities commonly associated with soil-transmitted helminthiasis, it is difficult to assess the true burden due to these infections.

Objectives The goal guiding this Ph.D. project was to deepen the understanding of the epidemiology and burden of soil-transmitted helminthiasis among school-aged children from the Bulang ethnic minority group in P.R. China. The following objectives were pursued: (i) to evaluate the feasibility of deploying different tools for the assessment of physical fitness in soil-transmitted helminth-endemic settings; (ii) to monitor changes in physical fitness, strength and anthropometric measurements over a six-month period among treated children and their untreated peers; (iii) to predict and visualize the change in physical fitness of school-aged children due to soil-transmitted helminth infections over a 1-month period across P.R. China; (iv) to assess the efficacy of triple-dose albendazole and study soil-transmitted helminth re-infection patterns after deworming; and (v) to estimate the odds of re-infection with soil-transmitted helminths for different natural nutritional statuses and types of nutritional supplementation.

Methods For the field studies, parasitological examination of stool samples was performed. The Kato-Katz technique was used to identify the eggs of *A. lumbricoides*, hookworm, *T. trichiura* and other helminths, while the Baermann technique was used to identify the

larvae of *S. stercoralis*. In addition, each stool sample was visually inspected for *Taenia* spp. proglottids. Physical fitness was estimated with the 20-m shuttle run test and physical strength was assessed with the grip strength and standing broad jump tests. Anthropometric measurements, including body height and weight and sum of skinfolds, and haemoglobin level were also recorded. Physical fitness and strength scores, anthropometric measurements, and haemoglobin level were expressed as means, and compared among children of distinct soil-transmitted helminth infection status and intensity. For the prediction and visualization exercise, the change in physical fitness over 1 month across P.R. China was predicted over a smooth surface of soil-transmitted helminth risk. Maps, with lower and upper boundaries of the predicted values as well as population-adjusted estimates, were further created. Finally, for the systematic review, the odds of re-infection with soil-transmitted helminths for different natural nutritional statuses and types of nutritional supplementation were estimated and qualitative content analysis was conducted for all studies included in the review.

Results In a cross-sectional survey, the maximum aerobic capacity in 1 min of exhaustive exercise (VO_2 max estimate) of *T. trichiura*-infected children was $1.9 \text{ ml kg}^{-1} \text{ min}^{-1}$ lower than that of their non-infected counterparts ($P=0.01$). Until exhaustion, *T. trichiura*-infected children had completed six 20-m laps less ($P<0.01$). No significant association between anthropometric indicators and infection with any soil-transmitted helminth species could be established.

In a randomised controlled trial, which investigated the effects of triple-dose albendazole on physical fitness of school-aged children, baseline prevalences of *T. trichiura*, *A. lumbricoides*, hookworm, and *S. stercoralis* were 94.5%, 93.3%, 61.3%, and 3.1% respectively, with more than half harboring triple-species infections. During the course of the trial, rapid re-infection with *A. lumbricoides* was observed and low cure rate was achieved with *T. trichiura* infections. Children receiving triple-dose albendazole scored slightly higher values in physical fitness and strength scores, anthropometric measurements, and haemoglobin level than placebo recipients, but the difference lacked statistical significance. The increase in VO_2 max estimate from baseline was $1.6 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P=0.02$) less and the increase in the number of 20-m laps completed from baseline was five 20-m laps ($P=0.04$) less for *T. trichiura*-infected children compared to their non-infected peers. In addition, children with low infection intensity of *T. trichiura* and hookworm had consistently more increase in the VO_2 max estimate from baseline than their peers with high infection intensity of all soil-transmitted helminths (range: $1.9\text{--}2.1 \text{ ml kg}^{-1} \text{ min}^{-1}$; all $P < 0.05$).

In the systematic review, multi-micronutrients seemed to have the clearest effect with regards to lowering re-infection rates and intensity of soil-transmitted helminths, whereas consumption of zinc or vitamin A alone might have a negative impact on these two outcomes measures. With regards to the natural nutrition status of the host, the general trend observed was that individuals with poor nutrition status suffered higher re-infection rates and intensities when compared to their well-nourished peers. Overall, only fifteen studies met our inclusion criteria and majority of them were of low quality.

Conclusions/significance The negative associations observed between *T. trichiura* infections and physical fitness among school-aged Bulang children in Yunnan suggests that the current burden estimate of soil-transmitted helminth infections, in particular *T. trichiura* infections, might be underestimated and there are still subtle and hidden morbidities to be quantified. A paradigm shift is needed to further understand the burden of soil-transmitted helminth infections as the presence of co-infections and co-morbidities add layers of complexity to the task. Finally, the epidemiological findings on soil-transmitted helminthiasis from this thesis highlight that a national soil-transmitted helminth control programme is overdue and urgently needed as P.R. China further develops into a global powerhouse. With many of their rural communities starting to have their hands on the first rung of the development ladder, P.R. China seems to be in a good position to set a leading example on how to control and eliminate soil-transmitted helminthiasis, and possibly other neglected tropical diseases, for developing countries around the world.

3. Zusammenfassung

Hintergrund Die drei am weitesten verbreiteten Boden-übertragenen Helminthen sind *Ascaris lumbricoides* (Spulwurm), *Trichuris trichiura* (Peitschenwurm), und die Hakenwürmer *Ancylostoma duodenale* und *Necator americanus*. Zusammen verursachen sie zirka 1 Milliarde Helmintheninfektionen bei Menschen und gefährden weltweit in etwa 5 Milliarden Menschen welche in Risikogebieten leben. *Strongyloides stercoralis* ist eine weniger weitverbreitete Boden-übertragene Helminthenart als die oben genannten, ist aber dennoch von grosser Bedeutung für die öffentliche Gesundheit. Die weltweite Prävalenz von *S. stercoralis* wird auf 30-100 Million geschätzt. Die Bevölkerungsgruppen, welche am meisten unter den Boden-übertragenen Helminthen leiden, sind oft arm, leben mit weniger als 2 US\$ pro Tag und haben unzureichende sanitäre Anlagen und hygienische Verhaltensweisen. Trotz der niedrigen nationalen Prävalenz von Helmintheninfektionen in der Volksrepublik China, existieren weiterhin Infektionshotspots, vor allem unter den Angehörigen ethnischer Minderheiten in abgelegenen ländlichen Gebieten.

Auf Grund der chronischen und subtilen Art der Morbiditäten welche durch Helminthen am häufigsten verursacht werden, ist es schwierig die wahre Bürde dieser Infektionen einzuschätzen. Infektionen durch die einzelnen Parasitenarten können verschiedenartige Symptome von Abdominal Schmerzen bis hin zur Anämie verursachen sowie eine Verzögerung der kognitiven Entwicklung bewirken. Bei Infektionen mit *S. stercoralis* wurden schwere Bauchschmerzen so wie Blut im Stuhl als Symptome berichtet.

Zielsetzung Das Übergreifende Ziel dieser Doktorarbeit war es, das Verständnis der Epidemiologie so wie der Bürde von Helmintheninfektionen bei Schulkindern der ethnischen Minderheit Bulang in der Volksrepublik China zu vertiefen. Dabei wurden die folgenden Zielsetzungen verfolgt: (i) Evaluieren der Durchführbarkeit der Entwicklung von verschiedenen Methoden zur Beurteilung der Fitness von Schulkindern in Helminthen-endemischen Gebieten; (ii) Das Überwachen der Veränderungen der Fitness, der Kraft und der anthropometrischen Messungen über einen Zeitraum von sechs Monaten hinweg in behandelten und unbehandelten Kindern; (iii) Das Vorhersagen und Visualisieren der durch Helmintheninfektionen verursachten Veränderungen der Fitness von Kindern im Schulalter in der gesamten Volksrepublik China über einen Zeitraum von einem Monat; (iv) Das Evaluieren der Effektivität von drei Dosen Albendazol und das Untersuchen der Reinfektionsmuster durch Boden-übertragene Helminthen nach einer Entwurmung; und (v)

Das Einschätzen der Wahrscheinlichkeit von Reinfektionen mit Boden-übertragenen Helminthen abhängig von verschiedenen natürlichen Ernährungszuständen sowie verschiedenen Arten von Nahrungsergänzungen.

Methoden Für die Feldstudien wurden parasitologische Stuhluntersuchungen durchgeführt. Die Kato-Katz Methode wurde verwendet um Eier von *A. lumbricoides*, Hakenwürmer, *T. trichiura* und anderen Helminthen zu identifizieren. Zudem wurde die Baermann Methode verwendet um Larven von *S. stercoralis* zu finden. Des weiteren wurden die Stuhlproben auch makroskopisch auf Proglottiden der Bandwürmer *Taenia* spp. untersucht. Die Fitness wurde mittels des “20-m shuttle run Tests“ gemessen und die physische Kraft durch Griffkraftmessung und den Standweitsprung evaluiert. Anthropometrische Messungen, einschliesslich der Körpergrösse, des Körpergewichtes und der Summe der Hautfaltendicke, sowie der Hämoglobinspiegel wurden ebenfalls erfasst. Die Mittelwerte der Fitnessmessungen, Kraftmessungen, anthropometrischen Messungen und des Hämoglobinspiegels wurden berechnet und zwischen Kindern mit distinkt unterschiedlichen Helmintheninfektionsstatus und Infektionsintensitäten verglichen. Für das Vorhersagen und Visualisieren der Infektionen wurde die Veränderung der Fitness bei Kindern der gesamten Volksrepublik China über einen Monat hinweg mit Hilfe einer smooth surface prediction des Helmintheninfektionsrisikos errechnet. Des Weiteren wurden Karten mit Unter- und Obergrenzen der Vorhersagewerte sowie mit Populations-normalisierten Berechnungen erstellt. Zuletzt wurde mittels einer systematischen Literaturanalyse die Wahrscheinlichkeit von Reinfektionen durch Boden-übertragene Helminthen abhängig von verschiedenen Ernährungszuständen und Arten von Nahrungsergänzungen evaluiert und eine qualitative Inhaltsanalyse wurde für alle in der Literaturanalyse eingeschlossenen Studien durchgeführt.

Resultate In der Querschnittstudie war die maximale aerobe Kapazität während 1 Minute anstrengender Bewegung (maximaler VO_2 Wert) von *T. trichiura*-infizierten Kindern $1.9 \text{ ml kg}^{-1} \text{ min}^{-1}$ niedriger als der von nicht-infizierten Kindern ($P=0.01$). *T. trichiura*-infizierte Kinder hatten zum Zeitpunkt ihrer Erschöpfung sechs 20-m Runden weniger absolviert ($P<0.01$) als ihre nicht infizierten Kameraden. Zwischen den anthropometrischen Indikatoren und der Infektion durch einer der Helminthen Arten wurde keine signifikante Assoziation gefunden.

In der randomisierten kontrollierten Doppelblindstudie welche die Auswirkung von drei Dosen Albendazol auf den Fitnesszustand von Schulkindern untersuchte, waren die Ausgangsprävalenzen für *T. trichiura*, *A. lumbricoides*, Hakenwürmer, und *S. stercoralis*:

94.5%, 93.3%, 61.3%, und 3.1% wobei mehr als die Hälfte der Studienteilnehmer gleichzeitig mit drei Helminthenarten infiziert waren. Während der Studie wurde eine rasche Reinfektionsrate mit *A. lumbricoides* beobachtet sowie eine lediglich geringe Heilungsrate von *T. trichiura* Infektionen. Kinder welche mit einer Dreifachdosis Albendazol behandelt wurden, erzielten eine leicht höhere Fitness, waren etwas stärker, hatten leicht höhere anthropometrische Werte und ihr Hämoglobinspiegel war höher als bei den mit Placebo behandelten Teilnehmern. Dieser Unterschied war jedoch nicht statistisch signifikant. Zwischen Baseline und follow-up hatten *T. trichiura*-infizierte Kinder einen um $1.6 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P=0.02$) geringeren Anstieg ihres maximalen VO_2 Wertes und die Zunahme der Anzahl absolvierter shuttle run Runden war um 5 geringer ($P=0.04$) als bei nicht-infizierten Kollegen. Zudem hatten Kinder mit einer niedrigen *T. trichiura* und Hakenwurm Infektionsintensität im Verlauf der Studie einen konsistent höheren Anstieg ihres maximalen VO_2 Wertes als Kinder mit hoher Infektionsintensität aller Helminthen (Bereich: $1.9\text{-}2.1 \text{ ml kg}^{-1} \text{ min}^{-1}$; all $P < 0.05$).

In der systematischen Literaturanalyse schienen multiple Mikronährstoffe den eindeutigsten positiven Effekt auf die Reinfektionsrate und Infektionsintensität von Bodenübertragenen Helminthen zu haben wohingegen die Einnahme von Zink und Vitamin A alleine negative Folgen auf beide Indikatoren zu haben scheint. Im Hinblick auf den natürlichen Ernährungszustand des Wirtes, haben Individuen mit einem schlechteren natürlichen Ernährungszustand mit höheren Reinfektionsraten und Infektionsintensitäten zu kämpfen als gut genährte Individuen. Insgesamt haben lediglich fünfzehn Studien unsere Einschlusskriterien für die Literaturanalyse erfüllt und die Meisten davon waren von niedrigerer Qualität.

Schlussfolgerung / Bedeutung: Die negative Assoziation zwischen einer *T. trichiura* Infektion und der Fitness von Bulang Schulkindern in Yunnan weist darauf hin, dass die derzeitigen Schätzung der Bürde von Helmintheninfektionen und speziell von Infektionen durch *T. trichiura*, vermutlich unterschätzt werden. Zudem weisen diese Daten darauf hin, dass es eventuell noch weitere subtile und verborgene Morbidität zu quantifizieren gibt. Ein Paradigmenwechsel ist notwendig um die Bürde von Helmintheninfektionen besser zu verstehen da das Vorkommen von Co-Infektionen so wie Co-Morbidität dem ganzen Vorhaben weitere Komplexität verleiht. Zu guter Letzt zeigen die epidemiologischen Ergebnisse dieser Dissertation, dass ein Nationales Helminthenkontrollprogramm in der aufstrebenden Grossmacht China überfällig und dringend notwendig ist. Mit den vielen

ländlichen Gebieten welche bereits beginnen die Entwicklungsleiter hinauf zu steigen, ist die Volksrepublik China hervorragend situiert als leitendes Beispiel für die Kontrolle und Elimination von Helminthen und eventuell auch anderer Vernachlässigter Tropenkrankheiten in Entwicklungsländern auf der ganzen Welt voranzugehen.

4. 摘要

背景 世上最普遍的土源性蠕虫有蛔虫，鞭虫和钩虫。全球，有十亿人口被这些蠕虫感染，另外有五十亿人有被感染的风险。线虫虽然没那么普遍，但还是有大约三千万到一亿人被感染。被蠕虫感染的人群通常生活在非常贫困和不卫生的环境中。在中国，蠕虫在全国的感染率虽然已经下降，但是还是有很多的高度感染集中在生活较贫困的少数民族人群里。据文学记载，蠕虫病能导致腹痛，贫血，影响智力的发展和粪便出血。由于蠕虫病的症状既慢性又不具体，所以想真正了解它的疾病负担并不容易。

目标 这博士论文的主要目标是想更深一层地去了解土源性蠕虫在云南布朗族学生里的流行趋势和蠕虫病的疾病负担。具体的目标包括：1) 评估测试体能的各种方法的可行性，并找出一个合适的方法能在拥有蠕虫病的环境里使用；2) 在六个月里，监测学生的体能，身高，体重，皮脂和血红蛋白量；3) 预测蠕虫病在全中国导致的体能减少；4) 评估阿苯达唑三倍剂量对蠕虫病的治愈率和了解各土源性蠕虫重复感染的方式；和 5) 预测不同营养状态和营养补充导致蠕虫病重复感染的可能性。

实验方法 我们利用加藤滕法来辨认蛔虫，鞭虫，钩虫和其它蠕虫的虫卵，并用贝尔曼技巧来辨认线虫的幼虫。同时，我们也在粪样中检查有没有绦虫的结片。我们用二十米来回跑来评估学生的体能，学生的实力则用握力器和跳远来评估。除了体能和实力，我们也测量了学生的身高，体重，皮脂和血红蛋白量。之后，我们则统计不同蠕虫感染率和感染度对这些指标的影响。我们也预测蠕虫病在全中国导致的体能减少。最后，我们预测了不同营养状态和营养补充导致蠕虫病重复感染的可能性，并对现有的营养学和蠕虫病文献内容做了性质上的分析。

成绩 在一次横断调查中，我们发现拥有鞭虫的学生的肺活量比没有鞭虫的学生要少 $1.9 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P=0.01$)。在二十米来回跑里，拥有鞭虫的学生也少跑了一百二十米 ($P<0.01$)。至于身高，体重，皮脂和血红蛋白量，我们并没有找出具有统计学意义的结果。另外，在一次随机对照试验中，我们发现鞭虫，蛔虫，钩虫和线虫在布朗族学生群里的感染率分别是 95%，93%，61%和 3%。在六个月的观测期里，我们不但发现蛔虫重复感染的几率很高，还发现阿苯达唑三倍剂量对鞭虫的治愈率偏低。虽然吃药的学生的体能和营养状态指标比没吃药的学生高，但这些结果并没有有统计学意义。不过，我们又再次发现拥有鞭虫的学生的体能并没有比没有鞭虫的学生好。拿底线

的成绩做参考，拥有鞭虫的学生的肺活量在随访时进步比没有鞭虫的学生少 $1,6 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P=0.02$)。拥有鞭虫的学生的跑步距离进步也少了一百米 ($P<0.04$)。拥有低度鞭虫和钩虫感染的学生的体能也明显地比拥有高度蛔虫，钩虫和鞭虫感染的学生来得好。最后，当我们将系统性地对营养学和蠕虫病文献内容做了性质上的分析时，我们发现多种维生素的补充品能减少蠕虫病重复感染的可能性。单倍锌或维生素A则增加了蠕虫病重复感染的可能性。与营养不充份的人比较，营养充份的人得蠕虫病重复感染的可能性要底些。

结论 鞭虫对学生体能的负面影响是值得我们更仔细地调查。我们需要更敏感，更好的测试方法来断定土源性蠕虫的疾病负担。蠕虫病在少数民族里的高感染率突出了中国现正需要一个全国性控蠕虫病的方案。要是控制蠕虫病成功，中国很有可能成为其它患有蠕虫病的发展中国家学习的榜样。

5. Introduction

In 1957, Norman R. Stoll, who had published the first systematic account of the worldwide prevalence of human intestinal helminth infection in 1947 (Stoll 1947), posed another question for the “wormy world”: “How approach the assignment of a consideration of the problem of helminthic infection as the cause of disability and disease in the tropics?” (Stoll 1957). This question remains pertinent, as intestinal helminths continue to be the most prevalent parasitic infections of humans (Hotez *et al.* 2007, 2008). In this Ph.D. thesis, a series of studies were conducted to determine the infection dynamics of soil-transmitted helminths, and to deepen our understanding of their impact on physical fitness and anthropometric indicators among school-aged children from the Bulang ethnic minority in Yunnan, People’s Republic of China (P.R. China). Focus is placed mainly on *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Ancylostoma duodenale* and *Necator americanus* (hookworms), and to a lesser extent, *Strongyloides stercoralis* (threadworm), and *Taenia* spp. (tapeworms; although not classified as soil-transmitted helminth, they are relevant for our study areas).

5.1. Biology and life cycle of selected intestinal helminths

5.1.1. Soil-transmitted helminths

The soil-transmitted helminths are a group of nematodes that are transmitted through soil contaminated with infectious faecal matter, with no non-human reservoir, and share certain aspects of their life cycles (Figure 5.1) (Bogitsh *et al.* 2011). Adult *A. lumbricoides* inhabit the small intestine and feed on partially digested food of the host. Eggs are passed with host faeces and after a period in moist soil exposed to optimal temperature and ambient oxygen levels for 2-4 weeks, the embryo inside fertilized eggs develops and molts in the shell to become an infective larva. Eggs accommodating infective larvae may remain viable in the soil for 2 years or longer. When these eggs are accidentally ingested, they hatch in the duodenum, where the larvae burrow out of the intestinal tract, pass through the liver, heart and lungs, and ascend the trachea before being swallowed back into the small intestine where they mature. This process requires approximately 3 months. Adult *A. lumbricoides* have 1-2 years of life expectancy inside the host.

Adult *T. trichiura* are found mainly in the colon, but sometimes in the appendix and rectum too. After being deposited in moist soil along with host faeces, an infective larva develops inside the egg within three to four weeks. When these eggs are ingested, the larvae hatch in the small intestine, penetrate the intestinal villi and finally, migrate back to the caecum for maturation. Adult *T. trichiura* implant themselves deeply into the colon submucosa, where they can remain about 2 years. There is little evidence available on their nutritional requirements in the host.

In contrast to these two parasites, hookworm larvae hatch and develop in the soil. Under optimal conditions, the infective third-stage larvae (L₃) can live up to 6 weeks in the soil. Infection occurs when these larvae penetrate the skin of the host, most often through hair follicles, pore, and skin abrasions. After migrating through the lymphatic system and heart, followed by a lung-throat passage similar to *A. lumbricoides*, the larvae embed in the small intestine for nourishment, via blood ingestion and maturation, which takes approximately 5-6 weeks. In the host, the mature hookworms have a long life expectancy of 3-4 years.

S. stercoralis is a less common soil-transmitted helminth than the species mentioned above, but still significant from a public health perspective. It has a complex life cycle involving three distinct phases: free living, parasitic and autoinfectious. In the free-living phase, adult *S. stercoralis* are non-parasitic and thrive in moist and warm soil, where copulation, hatching of eggs and the development of rhabditiform larvae occur. This cycle may continue uninterrupted if the optimal conditions remain. If not, the rhabditiform larvae will further develop into the infective filariform larvae, which can penetrate the host percutaneously, starting the parasitic phase. In the host, they follow a migration process similar to the hookworms before settling in the small intestine, where they mature into protandrogonous females, which produce fertilized eggs. During the autoinfectious phase, the rhabditiform larvae arising from these eggs may mature into the filariform form, which can re-penetrate the intestinal tract to start a new infection cycle without leaving the host.

8.1.2. Taeniasis

Taenia spp. are cestodes transmitted to humans mainly through the consumption of raw or undercooked meat containing their larvae (cysticerci). Maturation takes place in the small intestine, where the adult tapeworm can survive for years. The eggs or

proglottids (body segments containing eggs) are released in the faeces and ingested by animals, cattle in the case of *T. saginata* and pigs in the case of *T. solium*, in which they hatch, migrate to different locations including the muscles, and develop into cysts (Willingham 2010).

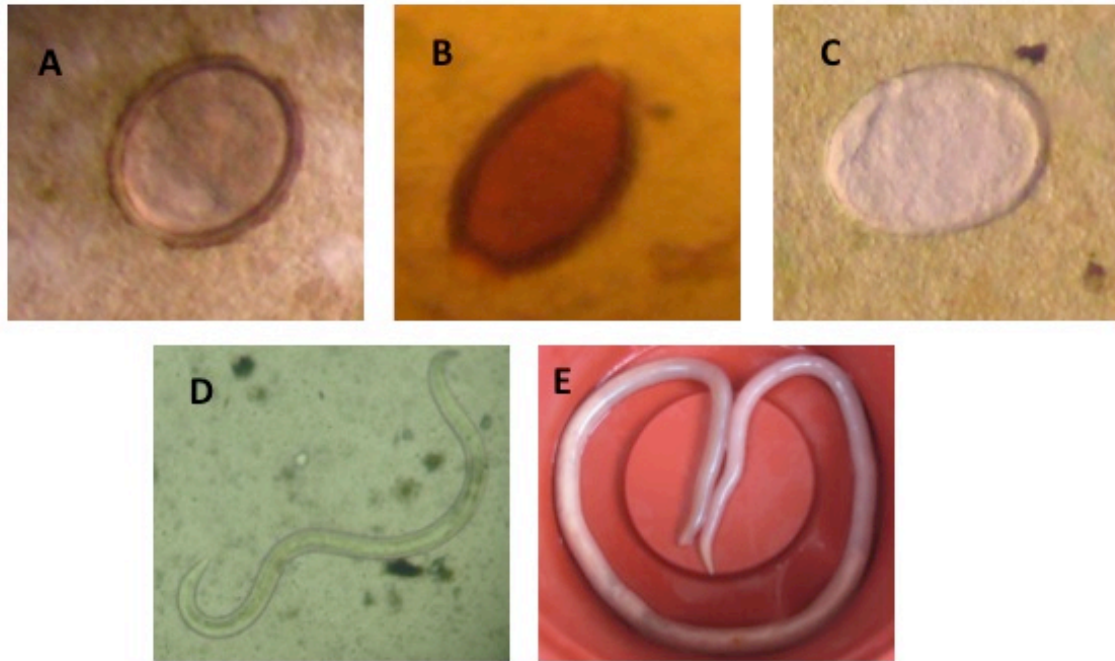


Figure 5.1. A collection of helminth eggs, larvae and adult worms from P.R. China.

A: *A. lumbricoides* egg; B: *T. trichiura* egg; C: hookworm egg; D: *S. stercoralis* larvae; E: Adult *A. lumbricoides*.

5.2. Epidemiology of soil-transmitted helminths

5.2.1. Global distribution of soil-transmitted helminths

The latest available estimates suggest that 807 million people are infected with *A. lumbricoides*, 604 million with *T. trichiura* and 576 million with hookworm (Hotez *et al.* 2008). Collectively, these soil-transmitted helminths are infecting around 1 billion people and putting approximately 5 billion people at risk of infection worldwide (Bethony *et al.* 2006; Brooker 2010; Pullan & Brooker 2012; WHO 2012). Among them, 875 million children are calculated to be at risk of infection, of whom 30% are preschool-aged and 70% school-aged (Barry *et al.* 2013). On the other hand, the global prevalence of *S. stercoralis* is estimated to be 30-100 million. Since the current field diagnostic

methods for this species are time- and labour-consuming, its diagnosis is often neglected and the figure could be obsolete and an underestimation (Olsen *et al.* 2009; Schär *et al.* 2013).

The largest numbers of soil-transmitted helminth infections occur in sub-Saharan Africa, South- and East Asia, the People's Republic of China (P.R. China), and the Americas (Brooker *et al.* 2006). Globally, there have been pronounced reductions in the prevalence of soil-transmitted helminth infections in the Americas as well as the high-income countries of Asia, but such a development has yet to begin in sub-Saharan Africa (de Silva *et al.* 2003; Chammartin *et al.* 2013b). The populations most affected by these parasitic worms are often impoverished, living on less than US\$ 2 per day (Hotez *et al.* 2007). In common, affected people have poor sanitation facilities and hygiene habits, low education and literacy rates, and belong to rural or marginalized urban communities where large inequities and barriers prevent them from accessing health care of good quality (Schratz *et al.* 2010; Steinmann *et al.* 2010).

5.2.2. Distribution of soil-transmitted helminths in P.R. China

Back in the 1960s, P.R. China had established an effective primary health care system consisting of “barefoot doctors”. These community health workers were minimally trained, visited rural villages and provided free treatments and health education for common diseases. Soil-transmitted helminth infection was one of the diseases controlled under the provision of such health care (Zhang & Unschuld 2008; Wang *et al.* 2012). However, the barefoot doctor system collapsed in the 1980s and soil-transmitted helminths remained rampant (Wu 2005). During a first nationwide survey on parasitic infections conducted between 1988 and 1992, approximately 1.5 million people in 30 provinces provided samples for faecal examination, and overall prevalences of 47%, 19%, and 17% were detected for *A. lumbricoides*, *T. trichiura*, and hookworm, respectively (Yu *et al.* 1994). The majority of these infections were of light intensity and it was estimated that more than 500 million cases of *A. lumbricoides* infections and about 200 million cases each of *T. trichiura* and hookworm infections were present in the country. Multiple parasitic infections were observed in 43% of the infected population (Xu *et al.* 1995). From 2001 to 2004, a second national survey was conducted to update the epidemiological information on parasitic infections in the country (Coordinating Office of the National Survey on the Important Human Parasitic Diseases 2005). The

study recorded progress with the control of soil-transmitted helminth infections and revealed the overall prevalences of *A. lumbricoides*, *T. trichiura* and hookworms to be 13%, 5% and 6%, respectively. Most infections were distributed in the provinces and autonomous regions of Hainan, Guangxi, Sichuan, Guizhou and Yunnan (Figure 5.2). A quarter of the infected survey participants had multiple infections, with six being the highest number of parasite species harboured by a single individual (Ohta & Waikagul 2007; Li *et al.* 2010).

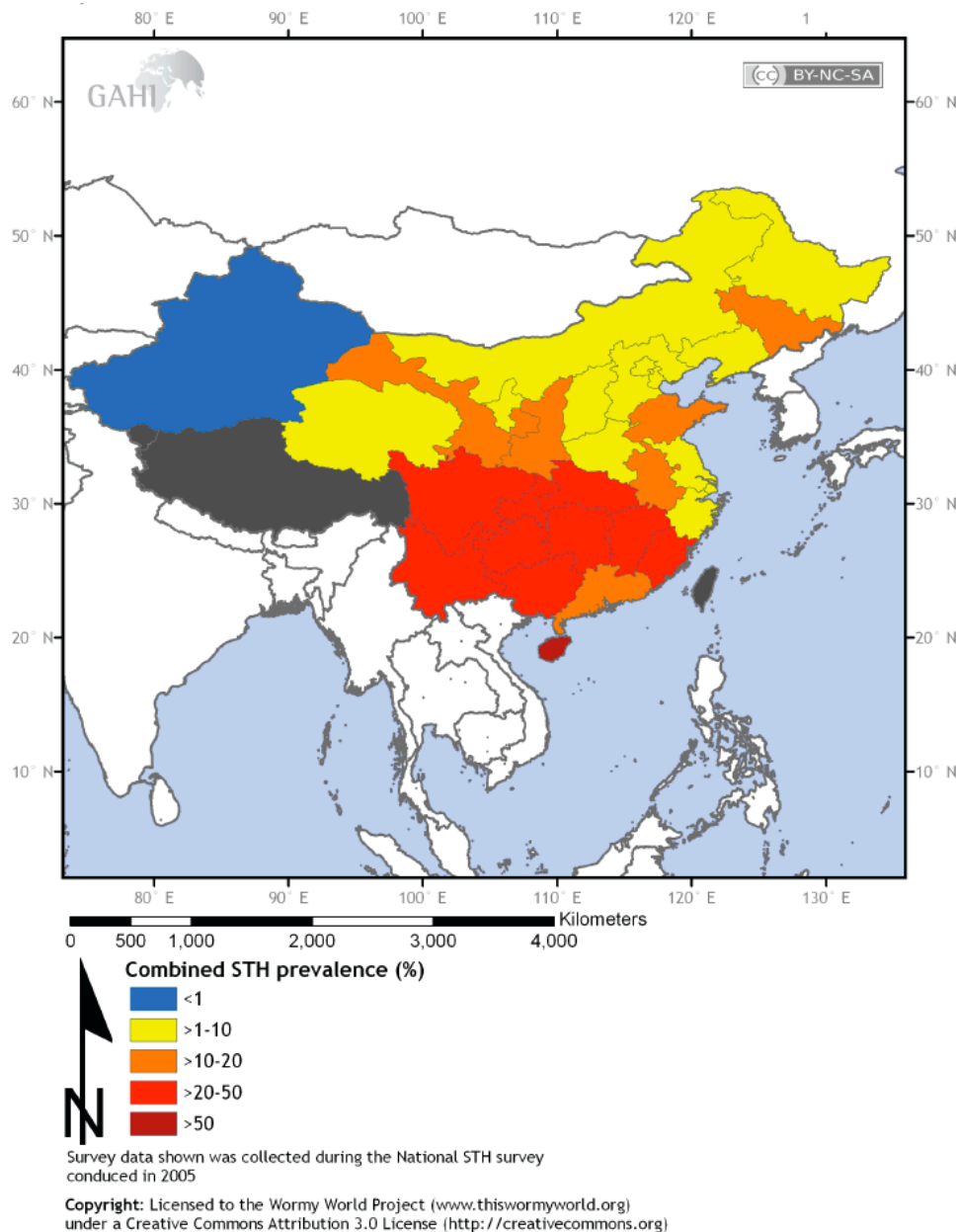


Figure 5.2. Distribution of soil-transmitted helminths across P.R. China.

STH: soil-transmitted helminth. Image obtained from the Wormy World Project (www.thiswormyworld.org; accessed: 26 July 2013).

Despite the prevalence reductions of soil-transmitted helminth infections observed in the second national survey, hotspots of these infections, particularly within ethnic minority groups residing in rural areas, still exist in P.R. China. Ethnic minority groups can be defined as “those with a social or cultural identity distinct from the dominant or mainstream society, which makes them vulnerable to being disadvantaged in the process of development” (ADB 1998). Indeed, these communities are often the poorest of the poor, with disease and mortality rates higher than that of the general population (Schratz *et al.* 2010). Officially, there are 56 distinct ethnic groups in P.R. China, of which 8% belong to 55 ethnic minority groups. Most of these ethnic minority communities live in the western, northern and southern parts of the country, such as Tibet, Inner Mongolia, Xinjiang, Guizhou, Guangxi, Sichuan and Yunnan provinces and autonomous regions. Many of these groups are located in remote mountainous regions. This geographic isolation, low population densities and poverty often result in inadequate provision and access to health care, education, basic infrastructure as well as transportation, translating into impaired economic perspectives (Hannum & Wang 2010). Studies in Bulang ethnic minority communities in Yunnan province revealed prevalences of *A. lumbricoides*, *T. trichiura* and hookworms to range from 61% to 96%, while the prevalence of *S. stercoralis* infections were between 3% and 12%. Multiple parasitic infections proved to be the norm rather than the exception as the proportion of individuals infected with three or more intestinal helminths was between 56% and 62% (Steinmann *et al.* 2007, 2008; Yap *et al.* 2013). Similarly, a survey of 141 rural communities in Guizhou and Sichuan provinces found that, respectively, 40% and 7% of school-aged children were infected with at least one type of soil-transmitted helminth species (Wang *et al.* 2012).

5.2.3. Dynamics of soil-transmitted helminth transmission

The basic reproductive number (R_0) is a summary indicator for the transmission of infectious diseases, including soil-transmitted helminths. The R_0 is defined as the average number of female offspring produced by a single adult female parasite that has achieved reproductive maturity, without density dependent constraints (Anderson & May 1991). Among the soil-transmitted helminths, the R_0 is highest for *T. trichiura* (4 to 6) and lowest for hookworms (2 to 3) (Brooker *et al.* 2006). Environmental factors play important roles in driving the transmission of soil-transmitted helminths and their survival is greatly influenced by land surface temperature (LST), aridity and altitude. In

terms of LST, experiments have shown that the infective stages develop optimally at temperatures between 28 and 32 °C, with development of *A. lumbricoides* and *T. trichiura* halting at below 5 °C and above 38 °C, and growth of hookworm larvae stopping at 40 °C (Brooker *et al.* 2006). The development of hookworm eggs is also impeded by cold temperatures (Chammartin *et al.* 2013a). Aridity and soil moisture are also important for the development of the infective stages, as faster development of the eggs and larvae is correlated with higher humidity and precipitation. The prevalence of soil-transmitted helminths tends to decrease to <2% in areas with an aridity index of <0.2, as embryonation of the eggs is inhibited at lower humidity (Pullan & Brooker 2012). Due to unfavourable temperatures and soil conditions, high altitude is detrimental to the survival of soil-transmitted helminths. In particular, higher altitudes have been shown to reduce *T. trichiura* infections (Flores *et al.* 2001; Chammartin *et al.* 2013a). On the other hand, seasonal variations are less likely to affect the overall transmission of soil-transmitted helminth infections because the life span of adult worms (1-10 years) is usually longer than the periods in the year when survival in the environment is constrained due to seasonal changes (Brooker *et al.* 2006).

Heterogeneity is another characteristic of soil-transmitted helminth epidemiology. In endemic communities, infections are typically aggregated and only a small percentage of individuals are heavily infected (Hotez *et al.* 2006). The reasons why some people seem predisposed to heavier and quicker re-infections than others are still unclear, but age is one of the main factors under consideration. The heaviest and most persistent infections of *A. lumbricoides* and *T. trichiura* occur in children aged 5-15 years, whilst a decline in prevalence and intensity is often observed in adults. These observations could be explained by a reduced exposure to contaminated environments among adults or indicate a steadily acquired protective immunity against soil-transmitted helminths over time (Woolhouse 1998). In contrast, for reasons still unclear, the intensity of hookworm infections shows a steady rise with age and peaks at adulthood instead (Gandhi *et al.* 2001; Bethony *et al.* 2002).

Multiparasitism is defined as the concurrent infection with two or more parasite species, and is a phenomenon commonly observed in individuals infected with soil-transmitted helminths (Steinmann *et al.* 2010). Field studies conducted in different countries have demonstrated the global significance of multiparasitism (Raso *et al.* 2004; Ohta & Waikagul 2007; Jardim-Botelho *et al.* 2008). When the extent of multiparasitism

was studied in a rural village in Côte d'Ivoire, 75% of study participants were infected with three or more parasite species concurrently, namely soil-transmitted helminths, *Schistosoma mansoni*, intestinal protozoa and *Plasmodium* spp. Out of the 500 individuals surveyed, 11 carried eight species; four harboured nine species and one person had 10 parasites. The younger age groups were also observed to carry less parasite species than the older age groups (Raso *et al.* 2004). In Brazil, a cross-sectional study revealed that almost half (48%) of the children sampled were co-infected with two parasitic infections and a further 19% were co-infected with *A. lumbricoides*, hookworm and *S. mansoni* (Jardim-Botelho *et al.* 2008). A field study performed in Vietnam, in a district within the Red River delta region, demonstrated that while 48% of the participants in a farming community harboured *A. lumbricoides*, hookworm and *T. trichiura* concurrently, 32% of them had dual infection with *A. lumbricoides* and *T. trichiura* (Needham *et al.* 1998).

When multiparasitism is present, synergism and antagonism in simultaneous infections are expected due to the different immune responses elicited by various parasites. Animal models have been established to study immune responses and co-infections. Mice that were usually susceptible to *Trichuris muris* were able to eliminate this intestinal helminth if co-infections with *S. mansoni* were present, as T helper 2 (Th2) responses, necessary for the expulsion of *T. muris*, were enhanced by *S. mansoni* (Curry *et al.* 1995). Immunity to *Strongyloides venezuelensis* was also observed to increase in mice with concurrent *S. mansoni* infections as these infections resulted in a build-up of cross-reactive antibodies against *S. venezuelensis* (Yoshida *et al.* 1999). Studies in humans are limited and they focus mainly on co-infections of intestinal helminths with *Plasmodium* spp. and the human immunodeficiency virus (HIV), respectively (Tian *et al.* 2009, 2012; Ivan *et al.* 2013; Mulu *et al.* 2013). In the case of co-infection with *Plasmodium* spp., the anti-inflammatory responses from intestinal helminths are thought to weaken the pro-inflammatory responses required for protective immunity to malaria, leading to persistent malarial infection. However, this process protects the host against severe inflammation due to malaria (Helmbj 2009; Mupfasoni *et al.* 2009; Supali *et al.* 2010). Similarly for co-infection with HIV, intestinal helminth infections are suggested to increase the host's susceptibility to HIV due to the resulting suppression of T helper 1 (Th1) responses (Brooker *et al.* 2004; Lewthwaite *et al.* 2005; Secor 2012).

Recognizing the complicated interplay of factors driving soil-transmitted helminth transmission, and their public health implications, would allow an improved understanding of the burden, diagnosis, treatment and control of these parasitic worms.

5.3. Burden of soil-transmitted helminths

5.3.1. Morbidities associated with soil-transmitted helminthiasis

Despite the near-ubiquitous nature of soil-transmitted helminths on a global scale, it is hard to assess the true burden due to these infections as limited research has been carried out to investigate the morbidities associated with them (Utzinger & de Savigny 2006; Nagpal *et al.* 2013). Furthermore, due to the chronic and subtle nature of most morbidities commonly associated with these parasites, communities might view the symptoms as a normal condition rather than one to be reversed. Hence, they might not report the symptoms if asked, or sense any need to seek treatment even though these infections might have long-term negative impacts on their health, physical and cognitive development as well as work productivity (Fenwick & Figenschou 1972; Stephenson *et al.* 2000; Crompton & Nesheim 2002; Jardim-Botelho *et al.* 2008; Eppig *et al.* 2010). Single-species infections with soil-transmitted helminths can cause symptoms ranging from abdominal pain, dysentery and physiological abnormalities in the intestinal tract to anaemia, pruritus, skin eruptions and impairment of the development of cognitive abilities. For *S. stercoralis* infections, which are especially neglected, symptoms such as severe abdominal pain, blood in the stool, cough and skin eruptions have been reported (Becker *et al.* 2011). The level of morbidity is strongly correlated to the numbers of worms the host harbours and individuals afflicted with multiparasitism arguably experience increased morbidities compared to people with single species infections (Brooker 2010). It is estimated that 300 million people worldwide suffer from severe morbidity due to soil-transmitted helminths, which also causes 10,000-135,000 deaths annually (Lustigman *et al.* 2012). Globally, years of life lost (YLLs) due to *A. lumbricoides* infections alone is at 204,111 (Lozano *et al.* 2012).

Soil-transmitted helminth infections may also lead to nutrient deficiency, which then manifests itself as immunodeficiency and impaired physical growth. Stephenson and colleagues (2000) have discussed mechanisms through which these parasitic worms cause malnourishment in the host, and one of the most important mechanisms was noted to be

anorexia. Worm infections often decrease the appetite of the infected individual, thus leading to a lowered dietary intake (Hadju *et al.* 1996). This results in deficiencies of essential nutrients, such as protein, iron, iodine, folate, zinc and vitamins A and B12. Moreover, intestinal inflammation due to such infections leads to decreased nutrient absorption. For example, poor fat absorption due to mucosal damage has been described in individuals infected with *A. lumbricoides*, hookworm and *T. trichiura*. *Taenia* spp. also compete with the host for nutrients, while obstruction and cellular damage in the liver caused by *A. lumbricoides* reduces the utilization of nutrients in the body (Stephenson *et al.* 2000). The presence of soil-transmitted helminths in the host can also increase nutrient loss, frequently in the form of blood loss in the faeces, or by causing diarrhoea (Stephenson *et al.* 1985).

Different nutrients are critical for specific immune functions and defenses. When an individual is deficient in any of them, a disruption in the immune integrity will result and eventually lead to increased susceptibility to infections (Gershwin *et al.* 2000). In terms of macronutrients, proteins are essential for antibody and interleukin formation (Malafaia *et al.* 2009), while lipids and carbohydrates play important roles in T cell production and function (Gershwin *et al.* 2000). In addition, a range of micronutrients has also been associated with healthy immune functions. Vitamin A guards the integrity of the epithelium in the respiratory and gastrointestinal tracts. A deficiency in vitamin A results in an increased risk for diarrhoeal infections (Scrimshaw & SanGiovanni 1997; Katona & Katona-Apte 2008). Both vitamins C and E are antioxidants, which scavenge free radicals and protect the body from oxidative damage. Vitamin E also enhances cell division and interleukin production in naive T cells (Carr & Frei 1999; Maggini *et al.* 2010). Zinc plays an important role in neutrophil and natural killer cell functions and complement activity. A deficiency in zinc suppresses antibody production and decreases the counts of T and B cells (Katona & Katona-Apte 2008; Maggini *et al.* 2010). Iron is associated with cell-mediated immunity as well as neutrophil function and hence, a lack of this element will also reduce immune defenses against infections (Katona & Katona-Apte 2008).

Several studies investigated the nutritional status of individuals infected with soil-transmitted helminths. In communities where food resources are scarce, stunting (low height-for-age), wasting (low body mass index-for-age) and anaemia are common indicators for undernourishment used for such assessments (FAO 2012). A 4-year

longitudinal study in rural South P.R. China provided evidence for the detrimental effects of both *T. trichiura* and *Schistosoma japonicum* infections on growth of children aged 10-12 years. In this study, anthropometric measurements and examination of stool samples for eggs of *S. japonicum*, *A. lumbricoides* and *T. trichiura* were also carried out. The study illustrated that shorter stature and lighter body weight were associated with infections by *T. trichiura* and *S. japonicum*, and elimination of *S. japonicum*, during the course of the study, improved anthropometric measurements (Zhou *et al.* 2007). On Pemba Island, Zanzibar, the association between hookworm infection intensity and haemoglobin concentration was found to be very strong. A hookworm infection of moderate intensity was estimated to double the iron requirements of the host and since the individuals in this population had low or no iron stores to meet these requirements, a decline in haemoglobin was seen even at the lowest levels of hookworm infection intensity. Other helminths, such as *T. trichiura* and *S. haematobium*, were also found to cause substantial iron loss in their hosts (Stoltzfus *et al.* 1997). Finally, anaemia and undernutrition, associated with multiparasitism were investigated in northern Rwanda. Children and adolescents, aged 5-20 years, were grouped into different multiparasitism profiles and their height, weight, haemoglobin concentration and *A. lumbricoides*, hookworms, *T. trichiura* and *S. mansoni* infection status were assessed. The study revealed that the levels of multiparasitism and infection intensities in this region were lower compared to other regions in sub-Saharan Africa. Subsequently, none of the multiparasitic infection profile was found to be significantly associated with stunting and being anaemic. However, the odds of being wasted were two times higher for children with simultaneous infection of at least two helminth species of moderate to high intensity as compared to children with mono-infection of low intensity or no infection (Mupfasoni *et al.* 2009).

Soil-transmitted helminth infections have also been shown to retard the cognitive development of infected children. Studies suggest that different intestinal helminth infections have distinct impacts on a child's cognitive development and children with multiparasitism suffer worse cognitive outcomes than children infected with a single helminth infection (Jardim-Botelho *et al.* 2008; Eppig *et al.* 2010). In the Philippines, Ezeamama and colleagues (2005) reported, in a cohort of children, prevalences of 92%, 74% and 46% for *T. trichiura*, *A. lumbricoides* and *N. americanus*, respectively. These infections were shown to be significantly associated with reduced performance in

cognitive tests. Another study in Jamaica illustrated that when *T. trichiura* infections were cleared in children, gains in auditory short-term memory, and the scanning and retrieval of long-term memory could be observed (Nokes *et al.* 1992). Similarly, in Indonesia, children were shown to have improved learning ability, eye-hand coordination, and cognitive test scores after *A. lumbricoides* infections were removed with chemotherapy (Hadidjaja *et al.* 1998). However, a recent randomized controlled trial in Sri Lanka concluded that cognitive test scores did not increase despite a reduction in soil-transmitted helminth infections due to successful school-based deworming (Ebenezer *et al.* 2013).

Studies attempting to correlate reduced school performance and physical fitness with soil-transmitted helminth infections have also been conducted. While children performed better in school after treatment of *T. trichiura* infections in a study in Jamaica (Simeon *et al.* 1995), another study in Guatemala showed that getting rid of *A. lumbricoides* infections did not lead to improvements in school performance (Watkins *et al.* 1996). In Kenya, studies have highlighted that reduced physical fitness was correlated with soil-transmitted helminth infections and treatment of these infections led to improved physical fitness in school-aged children (Stephenson *et al.* 1990, 1993; Bustinduy *et al.* 2011). However, another study in Côte d'Ivoire found no such correlations. Of note, the prevalence and intensity of soil-transmitted helminth infections in that study were very low (Müller *et al.* 2011).

Due to the limited and sometimes conflicting findings regarding the impact of soil-transmitted helminth infections on cognition, school performance and physical fitness, only morbidities such as anaemia due to hookworm, rectal prolapse due to *T. trichiura*, and bile duct or intestinal obstruction due to *A. lumbricoides*, have been well established and recognized within the scientific community (Lustigman *et al.* 2012). Indeed, under the Global Burden of Disease Study (GBD), the collective disability weight of intestinal nematode infections, which include soil-transmitted helminthiasis, is a mere 0.03 on a scale from 0 (perfect health) to 1 (death) (Salomon *et al.* 2012).

5.3.2. Estimating the global burden of soil-transmitted helminthiasis

Understanding the burden of a disease can be challenging, but its public health importance cannot be emphasized enough. Quantifying the burden of soil-transmitted helminth infections will allow evidence-based decision-making for the allocation of

limited health care resources (Nagpal *et al.* 2013). The first attempt to estimate the burden due to these parasitic worms using the concept of disability-adjusted life years (DALYs), the universal currency for quantifying ill-health, was performed by Murray and colleagues (1994) under the GBD Study in the 1990s (WB 1993). By defining a disability weight for each unique disease and then weighting the years lived with this disability, they were able to estimate the years lost due to the disability. DALYs have since been used effectively to quantify disabilities and pre-mature deaths associated with most diseases (Chan 1997). In 1990, all high intensity soil-transmitted helminth infections were given a disability weight of 0, but intestinal obstruction due to *A. lumbricoides*, rectal prolapse due to *T. trichiura*, and anaemia due to hookworm were assigned disability weights of 0.463, 0.116, and 0.024, respectively (Mathers *et al.* 2007). Based on these weights, *A. lumbricoides*, *T. trichiura*, and hookworms were calculated to cause a loss of 4.2, 0.9 and 3.9 million DALYs, respectively, giving a collective burden of 9.0 million DALYs (Murray & Lopez 1994; Murray *et al.* 1994). Within the community of experts on soil-transmitted helminthiases, a re-calculation was attempted on grounds of underestimation by the GBD Study. This gave rise to an estimate of 10.5, 6.4 and 22.1 million DALYs due to *A. lumbricoides*, *T. trichiura* and hookworm infections, respectively, resulting in a more substantial collective burden of 39.0 million DALYs (Bundy 1994). The much higher burden of hookworms was attributed to anaemia, especially in women of reproductive age. Murray and colleagues (1994) previously classified hookworm-induced anaemia under anaemia, instead of soil-transmitted helminth infections. Most of the DALYs were concentrated in the school-aged population, as they have the highest prevalence and infection intensity of soil-transmitted helminths, and a younger person with a permanent disability would incur more years lost than an older person (Chan 1997). The GDB was conducted again in 2010 to provide more comprehensive updates of the burden estimates for a wider range of diseases and conditions (Murray *et al.* 2012). In this study, *A. lumbricoides*, *T. trichiura* and hookworm were calculated to have 1.3, 0.6 and 3.2 million DALYs, respectively, giving a collective burden of 5.2 million DALYs (Lozano *et al.* 2012).

One of the limitations of the DALY estimates for soil-transmitted helminth infections is that it is almost impossible to assign accurate individual disability weights to each species due to the worldwide presence of multiparasitism. The use of quality-adjusted life years (QALYs) has thus been promoted to more comprehensively capture

the impact of soil-transmitted helminth infections on infected individuals. QALY values are calculated based on health-related quality of life (QoL) questionnaires administered to infected communities. QoL-related visual analogue scales and the short European quality of life (EuroQoL 5D) questionnaire are deemed to give a multidimensional illustration of the impact of a given condition, including soil-transmitted helminthiasis (Payne *et al.* 2009; King 2010). However, studies testing the validity of the concept of QALYs to account for morbidity due to soil-transmitted helminth infections are inadequate. These QoL questionnaires are very general and more suitable for the developed world and therefore, their applicability in rural communities, especially ethnic minority groups with certain strong cultural preferences, is questionable. Their effectiveness in detecting subtle and chronic morbidities due to soil-transmitted helminthiasis is still unclear. Indeed, when standardised QoL questionnaires were used in an attempt to assess self-rated quality of life and burden of soil-transmitted helminth infections in Yunnan province, the cultural unsuitability of these tools was noted. In such a case, the perception, and hence the reported outcome is not an accurate reflection of the reality (Ziegelbauer *et al.* 2010).

Before the morbidities associated with chronic soil-transmitted helminth infections are better defined, it will be difficult to give an accurate estimate of the public health burden due to these diseases. As much as we need an improved algorithm to calculate the public health burden of soil-transmitted helminth infections, we must also find new tools to assess morbidities, which are caused by or could potentially be associated with them.

5.3.3. Measuring physical fitness in children infected with soil-transmitted helminths

In this thesis, we hypothesize that a significant yet currently neglected effect of soil-transmitted helminth infections is a reduction of the physical fitness in infected children. Soil-transmitted helminth infections can cause nutrient loss and decreased food intake, leading to reduced growth in children (Stephenson *et al.* 2000; Crompton & Nesheim 2002). All these symptoms can arguably cause a depression in physical fitness levels (Stephenson *et al.* 1990). Hookworm-induced anaemia can also cause fatigue, shortness of breath and decreased energy that will translate into reduced physical fitness (Bustinduy *et al.* 2011). Physical fitness can be further stratified into the following main categories: cardiovascular endurance (aerobic), musculoskeletal strength, anaerobic power and capacity, agility, flexibility, and balance (Council of Europe 1983). Fitness

testing for an individual can be conducted in a physiology or sports science laboratory, where actual physiological markers, such as heart rate, blood lactate concentrations, oxygen uptake and power output, can be measured accurately. However, these techniques are expensive and difficult to perform in resource-constrained settings in rural communities of the developing world. Fortunately, field-based tests, are available and serve as good alternatives for school-based assessments of physical fitness in children infected with soil-transmitted helminths (Artero *et al.* 2011).

Cardiovascular endurance and musculoskeletal strength are the two main components of physical fitness being investigated in this thesis. For the assessments of cardiovascular endurance, both the 20-m shuttle run test (Léger *et al.* 1988), which estimates the maximum aerobic capacity within 1 min of exhaustive exercise (VO_2 max), and the 1-mile walk/run test (Beets & Pitetti 2006), which measures the shortest time possible required by an individual to cover 1 mile of distance, can be used. However, only the 20-m shuttle run test has strong evidence supporting its good test-retest reliability in adolescents (Artero *et al.* 2011). To measure musculoskeletal strength of the body, the handgrip strength test can be used for the upper body (España-Romero *et al.* 2008), trunk lift test for the mid body (Patterson *et al.* 1997), and the standing broad jump test for the lower body (Ortega *et al.* 2008). With the handgrip strength test, the child is asked to grip the hand span of a dynamometer as hard as possible and the strength of the grip will be measured. In the trunk lift test, the child, while lying face down, is asked to lift his/her upper body off the ground using the back muscles. The distance from the floor to the chin provides an indicator for the mid body strength. Under the standing broad jump test, the child, while standing, is instructed to jump as far forward as possible with both legs, and the distance jumped is then correlated to the amount of strength in the lower body. Among these tests, only the handgrip strength test has been shown to provide reliable results during repeated measurements (Artero *et al.* 2011). With regards to the other components of physical fitness, the more established tests for anaerobic capacity, agility, flexibility, and balance are the sprint fatigue test, the Illinois agility run, sit and reach, and beam walk, respectively (TopEndSports 2013). By combining the aforementioned physical fitness measurements with diagnostic tools for soil-transmitted helminths, the effect of these infections on the physical fitness of school-aged children can be investigated. Such findings will aid in the definition of the public health burden of soil-transmitted helminth infections.

5.4. Diagnosis, treatment and control

5.4.1. Diagnosis of soil-transmitted helminths

It is imperative to have reliable field-based diagnostic techniques for the accurate determination of the prevalence and intensity of soil-transmitted helminths in a community. Such tests are also indispensable for the monitoring and evaluation of chemotherapy and other health interventions against parasitic worms. According to guidelines from the World Health Organization (WHO), the diagnosis of soil-transmitted helminths should be made with the Kato-Katz technique (Katz *et al.* 1972; Montresor *et al.* 1998). It facilitates the detection of helminth eggs that infected individuals pass in their faeces. This technique involves the preparation of faecal thick smears on slides, followed by the microscopic examination and enumeration of helminth eggs. To obtain a standardised estimate of the infection intensity, expressed as eggs present per 1 g of stool (EPG), the raw egg counts are multiplied by a factor of 24 (Montresor *et al.* 1998). However, since only 41.7 mg of actual stool is used per thick smear, eggs are not homogeneously distributed throughout a stool sample and there is day-to-day and intra-day variation in egg output, the reliability of the Kato-Katz method is limited by its low sensitivity when a single stool sample is available or when low intensity infections are present (Booth *et al.* 2003; Knopp *et al.* 2008). To boost the sensitivity, the number of slides prepared from a single sample must be increased, or multiple stool samples collected (Knopp *et al.* 2008; Steinmann *et al.* 2008).

For the diagnosis of *S. stercoralis*, there is currently no simple and accurate tool available. However, the Baermann technique is the most frequently employed in parasitological field surveys as it does not require sophisticated laboratory materials and is less time consuming than culturing or immunological techniques (Knopp *et al.* 2008; Olsen *et al.* 2009). In the Baermann technique, the stool sample is placed on medical gauze in a glass funnel and covered with water. The whole set up is treated with artificial light directed at the bottom of the sample. After 2 hours, the lowest portion of the liquid is collected, centrifuged and the sediment subjected to microscopic examination for the larvae of *S. stercoralis*. This technique takes advantage of the fact that since the larvae of *S. stercoralis* are active and phototactic, they migrate out of the illuminated fecal sample into the water and settling at the bottom to be collected (Garcia & Bruckner 2001).

Other field-based diagnostic tools commonly employed for the diagnosis of soil-transmitted helminths are the ether-concentration method, the Koga agar plate methods (Koga *et al.* 1991), and the FLOTAC/Mini-FLOTAC techniques (Cringoli *et al.* 2010, 2013). Regarding the ether-concentration method, the stool sample is fixed, for example, with sodium acetate-acetic acid-formalin solution (SAF). After the addition of ether, the solution is centrifuged and the sediment examined for parasite eggs and larvae (Glinz *et al.* 2010). For the Koga agar plate method, the stool sample is placed in the middle of an agar plate and the closed petri dish is incubated in a humid chamber for 48 hours at ambient temperature before being rinsed, for example with 10% acetyl formalin solution. The eluent is centrifuged and the sediment examined under a microscope for the larvae of hookworm and *S. stercoralis* (Koga *et al.* 1991). The FLOTAC technique enables the sediment of a centrifuged stool sample to be examined under a microscope, after being re-suspended in a flotation solution of a specific density and separated in a flotation chamber of the FLOTAC apparatus. The technique allows for the quantification of parasite eggs (Utzinger *et al.* 2008) and has been shown to have higher sensitivity in detecting low-intensity soil-transmitted helminth infections than the Kato-Katz method (Knopp *et al.* 2009). Nonetheless, one of the major limitations of the FLOTAC technique is the centrifugation step, which makes it difficult to be employed in resource-constrained settings. To address this drawback, a Mini-FLOTAC apparatus, which eliminates the need for centrifugation, has been introduced and is currently being validated. First experiences with this apparatus have found it to be as sensitive as the Kato-Katz technique (Barda *et al.* 2013a, b).

One of the main challenges of soil-transmitted helminth diagnosis is the lack of sensitive tools for the detection of infections in communities with low prevalence and intensity. The current practice is to adopt a range of diagnostic tools and perform multiple stool sampling (Knopp *et al.* 2008; Steinmann *et al.* 2008). As the control of soil-transmitted helminths progress towards the elimination stage in the future, a single, sensitive and broad-spectrum diagnostic technique will have to be developed for surveillance purposes.

5.4.2. Chemotherapy against soil-transmitted helminths

Chemotherapy is an essential tool to control soil-transmitted helminth infections. The WHO advocates the use of preventive chemotherapy, where periodic administrations

of treatments are carried out in those population subgroups, at highest risk of disease, without prior diagnosis (WHO 2006). The rationale behind this strategy is to reduce infection intensity and hence, reduce or eliminate morbidities from chronic helminth infections (Gabrielli *et al.* 2011). Albendazole and mebendazole are the drugs of choice for community-based mass drug administration against soil-transmitted helminths (WHO 2006). Both drugs show high cure and egg reduction rates for *A. lumbricoides*, whereas albendazole is clearly more efficacious against hookworm than mebendazole, and have good safety profiles. On the other hand, neither drug shows satisfactory cure rates for *T. trichiura* infections, albeit with reasonable egg reduction rates (Keiser & Utzinger 2008). The WHO also recommends the use of levamisole and pyrantel pamoate against soil-transmitted helminths. Both are active against *A. lumbricoides* but only levamisole exhibits moderate efficacy against *S. stercoralis*, while pyrantel pamoate is preferably used to treat heavy hookworm infections. Praziquantel is commonly used against infections with *Taenia* spp., and other tapeworms. Ivermectin is the drug of choice against *S. stercoralis*, but since this drug is not commonly available except for lymphatic filariasis control, multiple doses of albendazole and mebendazole are often used to treat infections with this parasite (Utzinger & Keiser 2004; Keiser & Utzinger 2010).

Multiple dosing and combination therapy might prove to be valuable strategies against soil-transmitted helminth infections, as they could respectively offer increased efficacy and a wider spectrum of increased activity. Although novel anthelmintic drugs are urgently needed in anticipation of the development of drug resistance, it is also hoped that drug combinations could delay the emergence of drug resistance. Indeed, combinations of mebendazole, levamisole and pyrantel pamoate have outperformed single dosing of these drugs against soil-transmitted helminths (Utzinger & Keiser 2004). Furthermore, triple-dose regimens were shown to produce higher cure rates for hookworm and *T. trichiura* as compared to single dose regimens (Steinmann *et al.* 2011) and a combination of mebendazole and ivermectin against *T. trichiura* was also found to give higher cure and egg reduction rates as compared to a combination of albendazole and ivermectin, albendazole alone and mebendazole alone (Knopp *et al.* 2010).

As chemotherapy alone is not able to prevent re-infections, it is also important to have non-chemotherapeutic health interventions in place for a more comprehensive and sustainable control of intestinal parasitic infections. These non-chemotherapeutic

interventions should also have the capacity to treat undernutrition, impaired cognitive development and other deficits resulting from the condition (Hall 2007).

5.4.3. Non-chemotherapeutic control strategies

A lack of proper sanitation promotes the transmission of soil-transmitted helminthiasis by causing contamination of the environment. In many rural communities worldwide, the concept of sanitation remains elusive and open defecation is practiced widely, but given the right education and motivation, introduction and usage of low cost pit latrines, which can be subsequently improved, can have a protective effect against the parasitic worms (Brown *et al.* 2013). Indeed, a systematic review has summarised that the odds of being infected with *T. trichiura*, hookworm and *A. lumbricoides* were reduced by 42%, 40%, and 46%, respectively, when proper sanitation was available and used (Ziegelbauer *et al.* 2012). In Brazil, households with storm water drains or full waterborne sewage systems were compared to households with neither, and results showed that the prevalence of *A. lumbricoides* decreased by up to 40% when the level of community sanitation increased (Moraes *et al.* 2004). Furthermore, inadequate sanitation was also shown to be a risk factor for soil-transmitted helminth infections in Côte d'Ivoire (Schmidlin *et al.* 2013). Proper sanitation does not just reduce environmental contamination of faecal material but they also provide a sense of dignity and security, especially for women, within communities. In particular, their provision should be integrated with mass drug administration under school-based control programmes for soil-transmitted helminthiasis (Freeman *et al.* 2013).

In a world of globalization, cultural lines have blurred and in the mainstream populations, western medical principles and values have generally been adopted by individuals, in terms of health-seeking behaviours, and by the society, in terms of health systems. However, this is not the case with many ethnic minority groups. Within these communities, cultural beliefs remain strongly impressed on the minds of the people and together with other factors, such as religion, poverty, geographical isolation and political marginalization, often direct them towards certain health-seeking behaviours and hygiene habits. Individuals and their families who want to practice health choices that deviate from conventions can face difficulties and resistance (McMullin *et al.* 2005; Bóia *et al.* 2006; Muela Ribera *et al.* 2009; Vandemark *et al.* 2010). Health education and behavioural intervention can be useful tools in guiding the targeted populations to

understand the health issues they are facing and take individual measures against them. Likewise for soil-transmitted helminth infections, health education tools can help create awareness for these diseases, especially since symptoms are subtle, and get communities to adopt good hygiene habits, mainly hand washing with soap, wearing protective clothing, using latrines, and eating food and drinking water that have been properly processed, all of which can help reduce the transmission of soil-transmitted helminth infections. A recent study from Hunan province, P.R. China, illustrated how a comprehensive health education package has led to reductions in *A. lumbricoides* transmission (Bieri *et al.* 2013). The health education package, which aims to engage school-aged children and impress upon them proper knowledge, attitudes and practices towards soil-transmitted helminth infections, included an attractive cartoon video, drawing and essay competitions, and take-home brochures. The general applicability of this health education package remains to be validated in communities with higher prevalence of soil-transmitted helminth infections and from different cultural backgrounds.

The task of inducing behavioural change is challenging. The WHO has published a step-by-step guide for participatory hygiene and sanitation transformation (PHAST) (WHO 1998), which can provide the first steps in getting communities to make improvements to their own health. Briefly, the steps include (i) problem identification, where individuals share stories and their perceived health problems of their communities; (ii) problem analysis, where community health and hygiene practices are examined to understand how diseases spread; (iii) planning for solutions, where an action plan is devised to stop the spread of disease; (iv) selecting options, where the community selects for themselves the type of sanitation and hygiene behaviors they want to adopt; (v) planning for new facilities and behaviour change, where tasks are delegated to different members of the community to implement; and (vi) planning for monitoring and evaluation, where progress of the whole transformation is appraised. The guide focuses on participatory methods, which could embolden individuals, regardless of their sex, age, social class or educational level, to be involved in the health issues and decisions concerning their own communities. Such a process might prove to be more rewarding and sustainable in inducing behavioural change than simply a top-down approach where individuals are told to change their lifestyle and habits from strangers outside of their

communities. However, more field-based investigations on the applicability and impact of PHAST are needed to confirm this claim (Musabayane 2000).

More recently, another method, termed the community-led total sanitation (CLTS), was developed for encouraging change in sanitation behavior (Chambers & Kar 2008). This approach is similar to PHAST but has an added component of using shame or social stigma as a driver for promoting the adoption of improved sanitation behavior. The use of such a mechanism to induce behavioral change has generated controversies (Pattanayak *et al.* 2009; Bartram *et al.* 2012) and the longer-term impact of CLTS on hygiene and sanitation transformation remains to be determined (Schmidlin *et al.* 2013).

Finally, knowledge on the influence of culture and ethnicity on health choices is pivotal for the successful implementation of the aforementioned control strategies. In order for health interventions to not only improve knowledge but also leading to actual behavioural change, they have to be culture-sensitive, cater to the needs of these populations and gain local ownership. Providing a sense of empowerment to ethnic minority groups often proves to be as important as the science and technology used to address their health concerns (Eshel *et al.* 2008).

5.5. References

- ADB (1998) Policy on indigenous people. Manila, Asian Development Bank.
- Anderson HR, May RM (1991) *Infectious disease of humans: dynamics and control*, Oxford, Oxford University Press.
- Artero EG, España-Romero V, Castro-Piñero J, Ortega FB, Suni J, Castillo-Garzon MJ, Ruiz JR (2011) Reliability of field-based fitness tests in youth *Int J Sports Med* **32**, 159-169.
- Barda B, Zepherine H, Rinaldi L, Cringoli G, Burioni R, Clementi M, Albonico M (2013a) Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victoria. *Parasit Vectors* **6**, 220.
- Barda BD, Rinaldi L, Ianniello D, Zepherine H, Salvo F, Sadutshang T, Cringoli G, Clementi M, Albonico M (2013b) Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. *PLoS Negl Trop Dis* **7**, e2344.
- Barry MA, Simon GG, Mistry N, Hotez PJ (2013) Global trends in neglected tropical disease control and elimination: impact on child health. *Arch Dis Child* **98**, 635-641.
- Bartram J, Charles K, Evans B, O'Hanlon L, Pedley S (2012) Commentary on community-led total sanitation and human rights: should the right to community-wide health be won at the cost of individual rights? *J Water Health* **10**, 499-503.
- Becker SL, Sieto B, Silué KD, Adjossan L, Kone S, Hatz C, Kern WV, N'Goran EK, Utzinger J (2011) Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Negl Trop Dis* **5**, e1292.
- Beets MW, Pitetti KH (2006) Criterion-referenced reliability and equivalency between the PACER and 1-mile run/walk for high school students. *Measur Phys Educ Exer Sci* **3**, S21-S33.
- Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **367**, 1521-1532.
- Bethony J, Chen J, Lin S, Xiao S, Zhan B, Li S, Xue H, Xing F, Humphries D, Yan W, Chen G, Foster V, Hawdon JM, Hotez PJ (2002) Emerging patterns of hookworm infection: influence of aging on the intensity of *Necator* infection in Hainan Province, People's Republic of China. *Clin Infect Dis* **35**, 1336-1344.
- Bieri FA, Gray DJ, Williams GM, Raso G, Li YS, Yuan L, He Y, Li RS, Guo FY, Li SM, McManus DP (2013) Health-education package to prevent worm infections in Chinese schoolchildren. *N Engl J Med* **368**, 1603-1612.

- Bogitsh BJ, Carter CE, Oeltmann TN (2011) *Human Parasitology - third edition*, Elsevier Academic Press.
- Bóia MN, Carvalho-Costa FA, Sodré FC, Eyer-Silva WA, Lamas CC, Lyra MR, Pinto VL, Jr., Cantalice Filho JP, Oliveira AL, Carvalho LM, Gross JB, Sousa AL, Moraes TI, Bermudez-Aza EH, Martins EB *et al.* (2006) Mass treatment for intestinal helminthiasis control in an Amazonian endemic area in Brazil. *Rev Inst Med Trop São Paulo* **48**, 189-195.
- Booth M, Vounatsou P, N'Goran E K, Tanner M, Utzinger J (2003) The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing *Schistosoma mansoni* and hookworm co-infections in rural Côte d'Ivoire. *Parasitology* **127**, 525-531.
- Brooker S (2010) Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers - a review. *Int J Parasitol* **40**, 1137-1144.
- Brooker S, Bethony J, Hotez PJ (2004) Human hookworm infection in the 21st century. *Adv Parasitol* **58**, 197-288.
- Brooker S, Clements ACA, Bundy DAP (2006) Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol* **62**, 221-261.
- Brown J, Cairncross S, Ensink JH (2013) Water, sanitation, hygiene and enteric infections in children. *Arch Dis Child* **98**, 629-634.
- Bundy DAP (1994) Immunoepidemiology of intestinal helminthic infections. 1. The global burden of intestinal nematode disease. *Trans R Soc Trop Med Hyg* **88**, 259-261.
- Bustinduy AL, Thomas CL, Fiutem JJ, Parraga IM, Mungai PL, Muchiri EM, Mutuku F, Kitron U, King CH (2011) Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric. *PLoS Negl Trop Dis* **5**, e1213.
- Carr AC, Frei B (1999) Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am J Clin Nutr* **69**, 1086-1107.
- Chambers R, Kar K (2008) Handbook on community-led total sanitation. London, Plan International.
- Chammartin F, Scholte RG, Malone JB, Bavia ME, Nieto P, Utzinger J, Vounatsou P (2013a) Modelling the geographical distribution of soil-transmitted helminth infections in Bolivia. *Parasit Vectors* **6**, 152.
- Chammartin F, Scholte RGC, Guimarães LH, Tanner M, Utzinger J, Vounatsou P (2013b) Soil-transmitted helminth infection in South America: a systematic review and geostatistical meta-analysis. *Lancet Infect Dis* **13**, 507-518.
- Chan MS (1997) The global burden of intestinal nematode infections - fifty years on. *Parasitol Today* **13**, 438-443.

- Coordinating Office of the National Survey on the Important Human Parasitic Diseases (2005) [A national survey on current status of the important parasitic diseases in human population]. *Chin J Parasitol Parasit Dis* **23**, 332-340 (in Chinese).
- Council of Europe (1983) Testing physical fitness: Eurofit experimental battery. Strasbourg, Council of Europe.
- Cringoli G, Rinaldi L, Albonico M, Bergquist R, Utzinger J (2013) Geospatial (s)tools: integration of advanced epidemiological sampling and novel diagnostics. *Geospat Health* **7**, 399-404.
- Cringoli G, Rinaldi L, Maurelli MP, Utzinger J (2010) FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nat Protoc* **5**, 503-515.
- Crompton DWT, Nesheim MC (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr* **22**, 35-59.
- Curry AJ, Else KJ, Jones F, Bancroft A, Grencis RK, Dunne DW (1995) Evidence that cytokine-mediated immune interactions induced by *Schistosoma mansoni* alter disease outcome in mice concurrently infected with *Trichuris muris*. *J Exp Med* **181**, 769-774.
- de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L (2003) Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* **19**, 547-551.
- Ebenezer R, Gunawardena K, Kumarendran B, Pathmeswaran A, Jukes MC, Drake LJ, de Silva N (2013) Cluster-randomised trial of the impact of school-based deworming and iron supplementation on the cognitive abilities of schoolchildren in Sri Lanka's plantation sector. *Trop Med Int Health* **18**, 942-951.
- Eppig C, Fincher CL, Thornhill R (2010) Parasite prevalence and the worldwide distribution of cognitive ability. *Proc Biol Sci* **277**, 3801-3808.
- Eshel A, Moore A, Mishra M, Wooster J, Toledo C, Uhl G, Aguero LW (2008) Community stakeholders' perspectives on the impact of the minority AIDS initiative in strengthening HIV prevention capacity in four communities. *Ethn Health* **13**, 39-54.
- España-Romero V, Artero EG, Santaliestra-Pasias AM, Gutierrez A, Castillo MJ, Ruiz JR (2008) Hand span influences optimal grip span in boys and girls aged 6 to 12 years. *J Hand Surg Am* **33**, 378-384.
- FAO (2012) Undernourishment around the world in 2012. Rome, Food and Agriculture Organization of the United Nations.
- Fenwick A, Figenschou BH (1972) The effect of *Schistosoma mansoni* infection of the productivity of cane cutters on a sugar estate in Tanzania. *Bull World Health Organ* **47**, 567-572.
- Flores A, Esteban JG, Angles R, Mas-Coma S (2001) Soil-transmitted helminth infections at very high altitude in Bolivia. *Trans R Soc Trop Med Hyg* **95**, 272-277.

- Freeman MC, Ogden S, Jacobson J, Abbott D, Addiss DG, Amnie AG, Beckwith C, Cairncross S, Callejas R, Colford JM, Emerson PM, Fenwick A, Fishman R, Gallo K, Grimes J *et al.* (2013) Integration of water, sanitation, and hygiene for the prevention and control of neglected tropical diseases: a rationale for inter-sectoral collaboration. *PLoS Negl Trop Dis* **Epub ahead of print**.
- Gabrielli AF, Montresor A, Chitsulo L, Engels D, Savioli L (2011) Preventive chemotherapy in human helminthiasis: theoretical and operational aspects. *Trans R Soc Trop Med Hyg* **105**, 683-693.
- Gandhi NS, Jizhang C, Khoshnood K, Fuying X, Shanwen L, Yaoruo L, Bin Z, Haechou X, Chongjin T, Yan W, Wensen W, Dungxing H, Chong C, Shuhua X, Hawdon JM *et al.* (2001) Epidemiology of *Necator americanus* hookworm infections in Xiulongkan Village, Hainan Province, China: high prevalence and intensity among middle-aged and elderly residents. *J Parasitol* **87**, 739-743.
- Garcia LS, Bruckner DA (2001) *Diagnostic medical parasitology*, Washington D.C., American Society for Microbiology.
- Gershwin ME, German JB, Keen CL (2000) *Nutrition and Immunology: Principles and Practice.*, Humana Press.
- Glinz D, Silue KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, Rinaldi L, Cringoli G, N'Goran EK, Utzinger J (2010) Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. *PLoS Negl Trop Dis* **4**, e754.
- Hadidjaja P, Bonang E, Suyardi MA, Abidin SA, Ismid IS, Margono SS (1998) The effect of intervention methods on nutritional status and cognitive function of primary school children infected with *Ascaris lumbricoides*. *Am J Trop Med Hyg* **59**, 791-795.
- Hadju V, Stephenson LS, Abadi K, Mohammed HO, Bowman DD, Parker RS (1996) Improvements in appetite and growth in helminth-infected schoolboys three and seven weeks after a single dose of pyrantel pamoate. *Parasitology* **113 (Pt 5)**, 497-504.
- Hall A (2007) Micronutrient supplements for children after deworming. *Lancet Infect Dis* **7**, 297-302.
- Hannum E, Wang M (2010) Indigenous peoples, poverty and development: China: a case study in rapid poverty reduction Washington DC, World Bank.
- Helmby H (2009) Helminths and our immune system: friend or foe? *Parasitol Int* **58**, 121-127.
- Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J (2008) Helminth infections: the great neglected tropical diseases. *J Clin Invest* **118**, 1311-1321.
- Hotez PJ, Bundy DAP, Beegle K, Brooker S, Drake L, de Silva N, Montresor A, Engels D, Jukes M, Chitsulo L, Chow J, Laxminarayan R, Michaud C, Bethony J, Correa-Oliveira R *et al.* (2006) *Helminth infections: soil-transmitted helminth infections and*

- schistosomiasis*, Washington DC, The International Bank for Reconstruction and Development/The World Bank Group.
- Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, Savioli L (2007) Control of neglected tropical diseases. *N Engl J Med* **357**, 1018-1027.
- Ivan E, Crowther NJ, Mutimura E, Osuwat LO, Janssen S, Grobusch MP (2013) Helminthic infections rates and malaria in HIV-infected pregnant women on anti-retroviral therapy in Rwanda. *PLoS Negl Trop Dis* **7**, e2380.
- Jardim-Botelho A, Raff S, Rodrigues Rde A, Hoffman HJ, Diemert DJ, Corrêa-Oliveira R, Bethony JM, Gazzinelli MF (2008) Hookworm, *Ascaris lumbricoides* infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren. *Trop Med Int Health* **13**, 994-1004.
- Katona P, Katona-Apte J (2008) The interaction between nutrition and infection. *Clin Infect Dis* **46**, 1582-1588.
- Katz N, Chaves A, Pellegrino J (1972) A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Rev Inst Med Trop São Paulo* **14**, 397-400.
- Keiser J, Utzinger J (2008) Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* **299**, 1937-48.
- Keiser J, Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. *Adv Parasitol* **73**, 197-230.
- King CH (2010) Health metrics for helminthic infections. *Adv Parasitol* **73**, 51-69.
- Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, Marti H, Utzinger J (2008) Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis* **2**, e331.
- Knopp S, Mohammed KA, Speich B, Hattendorf J, Khamis IS, Khamis AN, Stothard JR, Rollinson D, Marti H, Utzinger J (2010) Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial. *Clin Infect Dis* **51**, 1420-1428.
- Knopp S, Rinaldi L, Khamis IS, Stothard JR, Rollinson D, Maurelli MP, Steinmann P, Marti H, Cringoli G, Utzinger J (2009) A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections. *Trans R Soc Trop Med Hyg* **103**, 347-354.
- Koga K, Kasuya S, Khamboonruang C, Sukhavat K, Ieda M, Takatsuka N, Kita K, Ohtomo H (1991) A modified agar plate method for detection of *Strongyloides stercoralis*. *Am J Trop Med Hyg* **45**, 518-521.
- Léger LA, Mercier D, Gadoury C, Lambert J (1988) The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci* **6**, 93-101.

- Lewthwaite P, Gill GV, Hart CA, Beeching NJ (2005) Gastrointestinal parasites in the immunocompromised. *Curr Opin Infect Dis* **18**, 427-435.
- Li T, He S, Zhao H, Zhao G, Zhu XQ (2010) Major trends in human parasitic diseases in China. *Trends Parasitol* **26**, 264-270.
- Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C *et al.* (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2095-2128.
- Lustigman S, Prichard RK, Gazzinelli A, Grant WN, Boatín BA, McCarthy JS, Basáñez MG (2012) A research agenda for helminth diseases of humans: the problem of helminthiasis. *PLoS Negl Trop Dis* **6**, e1582.
- Maggini S, Wenzlaff S, Hornig D (2010) Essential role of vitamin C and zinc in child immunity and health. *J Int Med Res* **38**, 386-414.
- Malafaia G, Serafim TD, Silva ME, Pedrosa ML, Rezende SA (2009) Protein-energy malnutrition decreases immune response to *Leishmania chagasi* vaccine in BALB/c mice. *Parasite Immunol* **31**, 41-49.
- Mathers CD, Ezzati M, Lopez AD (2007) Measuring the burden of neglected tropical diseases: the global burden of disease framework. *PLoS Negl Trop Dis* **1**, e114.
- McMullin JM, De Alba I, Chávez LR, Hubbell FA (2005) Influence of beliefs about cervical cancer etiology on Pap smear use among Latina immigrants. *Ethn Health* **10**, 3-18.
- Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L (1998) Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. Geneva, World Health Organization.
- Moraes LR, Cancio JA, Cairncross S (2004) Impact of drainage and sewerage on intestinal nematode infections in poor urban areas in Salvador, Brazil. *Trans R Soc Trop Med Hyg* **98**, 197-204.
- Muela Ribera J, Peeters Grietens K, Toomer E, Hausmann-Muela S (2009) A word of caution against the stigma trend in neglected tropical disease research and control. *PLoS Negl Trop Dis* **3**, e445.
- Müller I, Coulibaly JT, Fürst T, Knopp S, Hattendorf J, Krauth SJ, Stete K, Righetti AA, Glinz D, Yao AK, Pühse U, N'Goran E K, Utzinger J (2011) Effect of schistosomiasis and soil-transmitted helminth infections on physical fitness of school children in Côte d'Ivoire. *PLoS Negl Trop Dis* **5**, e1239.
- Mulu A, Maier M, Liebert UG (2013) Deworming of intestinal helminths reduces HIV-1 subtype C viremia in chronically co-infected individuals. *Int J Infect Dis* **17**, e897-901.
- Mupfasoni D, Karibushi B, Koukounari A, Ruberanziza E, Kaberuka T, Kramer MH, Mukabayire O, Kabera M, Nizeyimana V, Deville MA, Ruxin J, Webster JP, Fenwick

- A (2009) Polyparasite helminth infections and their association to anaemia and undernutrition in Northern Rwanda. *PLoS Negl Trop Dis* **3**, e517.
- Murray CJL, Lopez AD (1994) Quantifying disability: data, methods and results. *Bull World Health Organ* **72**, 481-494.
- Murray CJL, Lopez AD, Jamison DT (1994) The global burden of disease in 1990: summary results, sensitivity analysis and future directions. *Bull World Health Organ* **72**, 495-509.
- Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY *et al.* (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2197-2223.
- Musabayane N (2000) Management of rural drinking water supplies and waste using the participatory hygiene and sanitation transformation (PHAST) initiative in Zimbabwe. *Schriftenr Ver Wasser Boden Lufthyg* **105**, 81-87.
- Nagpal S, Sinclair D, Garner P (2013) Has the NTD community neglected evidence-based policy? *PLoS Negl Trop Dis* **7**, e2238.
- Needham C, Kim HT, Hoa NV, Cong LD, Michael E, Drake L, Hall A, Bundy DA (1998) Epidemiology of soil-transmitted nematode infections in Ha Nam Province, Vietnam. *Trop Med Int Health* **3**, 904-912.
- Nokes C, Grantham-McGregor SM, Sawyer AW, Cooper ES, Bundy DAP (1992) Parasitic helminth infection and cognitive function in school children. *Proc Biol Sci* **247**, 77-81.
- Ohta N, Waikagul J (2007) Disease burden and epidemiology of soil-transmitted helminthiasis and schistosomiasis in Asia: the Japanese perspective. *Trends Parasitol* **23**, 30-35.
- Olsen A, van Lieshout L, Marti H, Polderman T, Polman K, Steinmann P, Stothard R, Thybo S, Verweij JJ, Magnussen P (2009) Strongyloidiasis: the most neglected of the neglected tropical diseases? *Trans R Soc Trop Med Hyg* **103**, 967-972.
- Ortega FB, Artero EG, Ruiz JR, Vicente-Rodriguez G, Bergman P, Hagstromer M, Ottevaere C, Nagy E, Konsta O, Rey-Lopez JP, Polito A, Dietrich S, Plada M, Beghin L, Manios Y *et al.* (2008) Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. *Int J Obes (Lond)* **32 Suppl 5**, S49-57.
- Pattanayak SK, Yang JC, Dickinson KL, Poulos C, Patil SR, Mallick RK, Blitstein JL, Praharaj P (2009) Shame or subsidy revisited: social mobilization for sanitation in Orissa, India. *Bull World Health Organ* **87**, 580-587.
- Patterson P, Rethwisch N, Wiksten D (1997) Reliability of the trunk lift in high school boys and girls. *Measur Phys Educ Exer Sci* **1**, 145-151.

- Payne RJ, Turner L, Morgan ER (2009) Inappropriate measures of population health for parasitic disease? *Trends Parasitol* **25**, 393-395.
- Pullan RL, Brooker SJ (2012) The global limits and population at risk of soil-transmitted helminth infections in 2010. *Parasit Vectors* **5**, 81.
- Raso G, Luginbuhl A, Adjoua CA, Tian-Bi NT, Silue KD, Matthys B, Vounatsou P, Wang Y, Dumas ME, Holmes E, Singer BH, Tanner M, N'Goran E K, Utzinger J (2004) Multiple parasite infections and their relationship to self-reported morbidity in a community of rural Côte d'Ivoire. *Int J Epidemiol* **33**, 1092-1102.
- Salomon JA, Vos T, Hogan DR, Gagnon M, Naghavi M, Mokdad A, Begum N, Shah R, Karyana M, Kosen S, Farje MR, Moncada G, Dutta A, Sazawal S, Dyer A *et al.* (2012) Common values in assessing health outcomes from disease and injury: disability weights measurement study for the Global Burden of Disease Study 2010. *Lancet* **380**, 2129-2143.
- Schär F, Trostorf U, Giardina F, Khieu V, Muth S, Marti H, Vounatsou P, Odermatt P (2013) *Strongyloides stercoralis*: global distribution and risk factors. *PLoS Negl Trop Dis* **7**, e2288.
- Schmidlin T, Hürlimann E, Silué KD, Yapi RB, Hounghbedji C, Kouadio BA, Acka-Douabélé CA, Kouassi D, Ouattara M, Zouzou F, Bonfoh B, N'Goran EK, Utzinger J, Raso G (2013) Effects of hygiene and defecation behavior on helminths and intestinal protozoa infections in Taabo, Cote d'Ivoire. *PLoS One* **8**, e65722.
- Schratz A, Pineda MF, Reforma LG, Fox NM, Le Anh T, Tommaso Cavalli-Sforza L, Henderson MK, Mendoza R, Utzinger J, Ehrenberg JP, Tee AS (2010) Neglected diseases and ethnic minorities in the Western Pacific Region exploring the links. *Adv Parasitol* **72**, 79-107.
- Scrimshaw NS, SanGiovanni JP (1997) Synergism of nutrition, infection, and immunity: an overview. *Am J Clin Nutr* **66**, 464S-477S.
- Secor WE (2012) The effects of schistosomiasis on HIV/AIDS infection, progression and transmission. *Curr Opin HIV AIDS* **7**, 254-259.
- Simeon DT, Grantham-McGregor SM, Callender JE, Wong MS (1995) Treatment of *Trichuris trichiura* infections improves growth, spelling scores and school attendance in some children. *J Nutr* **125**, 1875-1883.
- Steinmann P, Du ZW, Wang LB, Wang XZ, Jiang JY, Li LH, Marti H, Zhou XN, Utzinger J (2008) Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. *Am J Trop Med Hyg* **78**, 760-769.
- Steinmann P, Utzinger J, Du ZW, Jiang JY, Chen JX, Hattendorf J, Zhou H, Zhou XN (2011) Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp.: a randomized controlled trial. *PLoS One* **6**, e25003.

- Steinmann P, Utzinger J, Du ZW, Zhou XN (2010) Multiparasitism: a neglected reality on global, regional and local scale. *Adv Parasitol* **73**, 21-50.
- Steinmann P, Zhou XN, Du ZW, Jiang JY, Wang LB, Wang XZ, Li LH, Marti H, Utzinger J (2007) Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and comparison of diagnostic methods. *PLoS Negl Trop Dis* **1**, e75.
- Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A (1993) Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr* **123**, 1036-1046.
- Stephenson LS, Latham MC, Kinoti SN, Kurz KM, Brigham H (1990) Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg* **84**, 277-282.
- Stephenson LS, Latham MC, Kurz KM, Miller D, Kinoti SN, Oduori ML (1985) Urinary iron loss and physical fitness of Kenyan children with urinary schistosomiasis. *Am J Trop Med Hyg* **34**, 322-330.
- Stephenson LS, Latham MC, Ottesen EA (2000) Malnutrition and parasitic helminth infections. *Parasitology* **121 Suppl**, S23-38.
- Stoll NR (1947) This wormy world. *J Parasitol* **33**, 1-18.
- Stoll NR (1957) Symposium on helminthic infections as the case of disability and disease; introduction. *Am J Trop Med Hyg* **6**, 399-401.
- Stoltzfus RJ, Dreyfuss ML, Chwaya HM, Albonico M (1997) Hookworm control as a strategy to prevent iron deficiency. *Nutr Rev* **55**, 223-232.
- Supali T, Verweij JJ, Wiria AE, Djuardi Y, Hamid F, Kaisar MM, Wammes LJ, van Lieshout L, Luty AJ, Sartono E, Yazdanbakhsh M (2010) Polyparasitism and its impact on the immune system. *Int J Parasitol* **40**, 1171-1176.
- Tian LG, Chen JX, Wang TP, Cheng GJ, Steinmann P, Wang FF, Cai YC, Yin XM, Guo J, Zhou L, Zhou XN (2012) Co-infection of HIV and intestinal parasites in rural area of China. *Parasit Vectors* **5**, 36.
- Tian LG, Steinmann P, Chen JX, Chen SH, Zhou XN (2009) HIV/AIDS, parasites and co-infections: publication patterns in China. *Parasit Vectors* **2**, 31.
- TopEndSports (2013) Fitness testing (<http://www.topendsports.com/testing/tests.htm>; accessed August 2013).
- Utzinger J, de Savigny D (2006) Control of neglected tropical diseases: integrated chemotherapy and beyond. *PLoS Med* **3**, e112.
- Utzinger J, Keiser J (2004) Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. *Expert Opin Pharmacother* **5**, 263-285.

- Utzinger J, Rinaldi L, Lohourignon LK, Rohner F, Zimmermann MB, Tschannen AB, N'Goran E K, Cringoli G (2008) FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. *Trans R Soc Trop Med Hyg* **102**, 84-90.
- Vandemark LM, Jia TW, Zhou XN (2010) Social science implications for control of helminth infections in Southeast Asia. *Adv Parasitol* **73**, 137-170.
- Wang X, Zhang L, Luo R, Wang G, Chen Y, Medina A, Eggleston K, Rozelle S, Smith DS (2012) Soil-transmitted helminth infections and correlated risk factors in preschool and school-aged children in rural Southwest China. *PLoS One* **7**, e45939.
- Watkins WE, Cruz JR, Pollitt E (1996) The effects of deworming on indicators of school performance in Guatemala. *Trans R Soc Trop Med Hyg* **90**, 156-161.
- WB (1993) World development report: investing in health. Washington D.C., World Bank.
- WHO (1998) PHAST step-by-step guide: A participatory approach for the control of diarrhoeal diseases. Geneva, World Health Organization.
- WHO (2006) Preventive chemotherapy in human helminthiasis: coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme managers. Geneva, World Health Organization.
- WHO (2012) Eliminating soil-transmitted helminthiasis as a public health problem in children. Geneva, World Health Organization.
- Willingham AL, Wu, H.W., Conlan, J., Satrijia, F. (2010) Combating *Taenia solium* cysticercosis in Southeast Asia an opportunity for improving human health and livestock production. *Advances in Parasitology* **72**, 235-266.
- Woolhouse ME (1998) Patterns in parasite epidemiology: the peak shift. *Parasitol Today* **14**, 428-434.
- Wu GL (2005) Medical parasitology in China: a historical perspective. *Chin Med J (Engl)* **118**, 759-761.
- Xu LQ, Yu SH, Jiang ZX, Yang JL, Lai LQ, Zhang XJ, Zheng CQ (1995) Soil-transmitted helminthiasis: nationwide survey in China. *Bull World Health Organ* **73**, 507-513.
- Yap P, Du ZW, Wu FW, Jiang JY, Chen R, Zhou XN, Hattendorf J, Utzinger J, Steinmann P (2013) Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in Yunnan, People's Republic of China. *Am J Trop Med Hyg* **89**, 23-31.
- Yoshida A, Maruyama H, Yabu Y, Amano T, Kobayakawa T, Ohta N (1999) Immune response against protozoal and nematodal infection in mice with underlying *Schistosoma mansoni* infection. *Parasitol Int* **48**, 73-79.

- Yu SH, Xu LQ, Jiang ZX, Xu SH, Han JJ, Zhu YG, Chang J, Lin JX, Xu FN (1994) Nationwide survey of human parasite in China. *Southeast Asian J Trop Med Public Health* **25**, 4-10.
- Zhang D, Unschuld PU (2008) China's barefoot doctor: past, present, and future. *Lancet* **372**, 1865-1867.
- Zhou H, Watanabe C, Ohtsuka R (2007) Impacts of dietary intake and helminth infection on diversity in growth among schoolchildren in rural south China: a four-year longitudinal study. *Am J Hum Biol* **19**, 96-106.
- Ziegelbauer K, Speich B, Mausezahl D, Bos R, Keiser J, Utzinger J (2012) Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. *PLoS Med* **9**, e1001162.
- Ziegelbauer K, Steinmann P, Zhou H, Du ZW, Jiang JY, Furst T, Jia TW, Zhou XN, Utzinger J (2010) Self-rated quality of life and school performance in relation to helminth infections: case study from Yunnan, People's Republic of China. *Parasit Vectors* **3**, 61.

6. Goals

The goal guiding this Ph.D. project was to deepen the understanding of the epidemiology and burden of soil-transmitted helminthiasis among school-aged children from the Bulang ethnic minority group in P.R. China. Particular emphasis was placed on re-infection dynamics and the effect of soil-transmitted helminth infection intensity and multiple species parasitic infections on physical fitness and nutritional indicators.

6.1. Specific objectives

To achieve this goal, the following objectives were pursued in a series of field studies, literature reviews and statistical analyses:

- To evaluate the feasibility of deploying different tools for the assessment of physical fitness in soil-transmitted helminth-endemic settings (Chapter 8).
- To monitor changes in physical fitness, strength and anthropometric measurements over a six-month period among treated children and their untreated peers (Chapter 9).
- To predict and visualize the change in physical fitness of school-aged children due to soil-transmitted helminth infections over a 1-month period across P.R. China (Chapter 10).
- To assess the efficacy of triple-dose albendazole and study soil-transmitted helminth re-infection patterns after deworming (Chapter 11).
- To estimate the odds of re-infection with soil-transmitted helminths for different natural nutritional statuses and types of nutritional supplementation (Chapter 12).

7. Study sites

The fieldwork described in this Ph.D. thesis was conducted in various Bulang communities located in Menghai county, Xishuangbanna Dai Autonomous prefecture, situated in Yunnan province, P.R. China (Figure 7.1). Similar in their geographical locations and socio-economic statuses, the eight Bulang villages are (i) Manguo new village (geographical coordinates: 21°45'09.01" N latitude and 100°18'47.20" E longitude, altitude: 1,550 m above sea level (asl)); (ii) upper Nanwen (21°46'02.15" N and 100°23'50.61" E; 1,650 m asl); (iii) lower Nanwen (21°46'34.02" N and 100°23'56.89" E; 1,550 m asl); (iv) Sandui (21°33'07" N and 100°19'34" E; 1,566 m asl); (v) Kongkan (21°32'34" N and 100°20'25" E; 1,195 m asl); (vi) Laozhai (21°31'37" N and 100°18'01" E; 1,399 m asl); (vii) Laonandong (21°33'28" N and 100°21'45" E; 1,188 m asl); and (viii) Mannuo (21°33'27" N and 100°23'53" E; 1,352 m asl).

The study sites border Myanmar and are mainly inhabited by the Bulang ethnic minority group. The mountainous area enjoys sub-tropical climate characterised by arid and cool winters and hot and rainy summers. Today, most children attend at least primary school, with boys commonly receive part of their education in Buddhist temple schools. Modern health care is lacking at village level, as only grassroots “doctors” with none or minimal formal medical training are present. Water supply in the villages is piped but water sources are not protected nor treated. Power outages are common. Sanitation infrastructure is generally unavailable and open defecation is widespread. Agriculture is the main source of income, with tea, sugarcane and bananas being important cash crops.

7.1. Ethics statement

The institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland) approved of all study protocols. Formal ethical clearance was further provided by the Ethikkommission beider Basel (EKBB, reference no. 144/11) and the Academic Board of the National Institute of Parasitic Diseases (IPD), Chinese Center for Disease Control and Prevention (China CDC) in Shanghai, P.R. China.

For each study, the village doctors, chiefs, and teachers were briefed on the aims of the study. With help from the teachers, the investigators further explained the procedures to the children. Written informed consent was obtained from

parents/guardians, whereas children assented orally. Data were kept anonymous. At the end of each study, albendazole was provided to all children, irrespective of their infection status and study participation, and ivermectin was also given to children diagnosed with *S. stercoralis*.

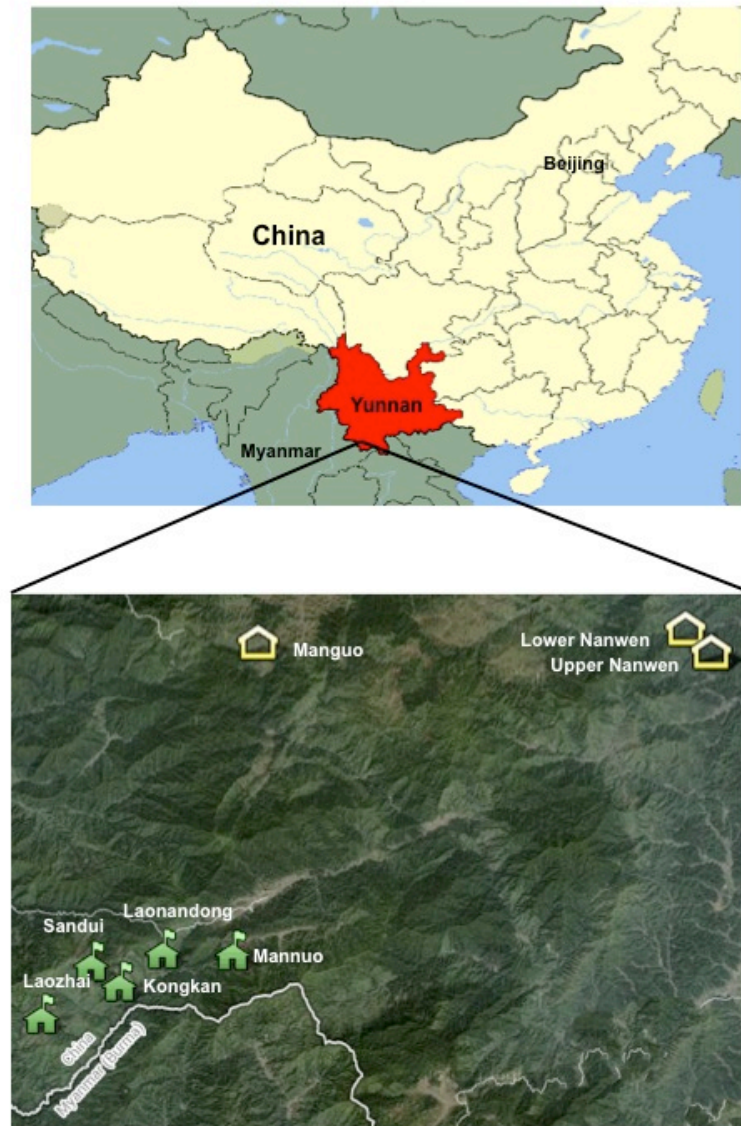


Figure 7.1. Location of the study sites.

Images adapted from Google Earth and Wikimedia Commons (accessed: 13 September 2013).

8. Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey

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8.1. Abstract

Background: Chronic soil-transmitted helminth (STH) infections have been associated with reduced physical fitness, but available evidence is limited. The aim of this cross-sectional survey was to assess the feasibility of measuring children's physical fitness and to relate it to STH infections. Our study was carried out among school-aged children of the Bulang ethnic group in rural southwest People's Republic of China (P.R. China). Standardized, quality-controlled methods were employed to determine STH infections (Kato-Katz technique), haemoglobin levels, anthropometry (body weight and height) and physical fitness (20-m shuttle run test).

Results: A compliance of 87% suggested good acceptance of the methods used. Among 69 children with complete data records, infection prevalence of *Trichuris trichiura*, *Ascaris lumbricoides* and hookworm were 81%, 44% and 6%, respectively. The maximum volume of oxygen that can be utilized within 1 min during exhaustive exercise (VO_2 max estimate) of *T. trichiura*-infected children was $1.94 \text{ ml kg}^{-1} \text{ min}^{-1}$ lower than that of their non-infected counterparts ($P = 0.005$). Until exhaustion, *T. trichiura*-infected children had completed 6.14 20-m laps less ($P = 0.004$). Additionally, the mean VO_2 max estimate of stunted children was lowered by $1.63 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P = 0.002$) and they completed 5.32 20-m laps less ($P = 0.001$) compared to children of normal stature. No significant association between stunting and infection with any STH species could be established.

Conclusions: Implementation of physical fitness tests in rural, resource-constraint settings is feasible. The physical fitness of children who are stunted or infected with STHs, particularly *T. trichiura*, is significantly impaired. We have launched a larger study and will determine the dynamics of school-aged children's physical fitness over a 7-month period after administration of anthelmintic drugs.

Keywords: soil-transmitted helminths, Kato-Katz technique, physical fitness, 20-m shuttle run test, anthropometry

8.2. Background

More than 1 billion people are parasitized by soil-transmitted helminths (STHs), namely *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) [1-4]. Taken together, STHs represent the most prevalent parasitic infection of mankind, but determining their exact geographical distribution, morbidity and global burden has proved difficult [5,6]. The highest infection rates are observed in the developing world. Control programmes there emphasize preventive chemotherapy, i.e. repeated administration of anthelmintic drugs to entire at-risk populations, particularly school-aged children [3,7,8]. Recognized common symptoms include abdominal pain, diarrhoea, anaemia, growth retardation and cognitive impairment. Hosts often carry more than one species simultaneously and may suffer from additive and/or multiplicative morbidity outcomes [9,10].

In the global burden of disease (GBD) study carried out between 2000 and 2004, high-intensity infections with STHs were given a zero disability weight (DW), while the cognitive impairment which can result from such infections was assigned DWs ranging from 0.024 to 0.463 [on a scale from 0 (perfect health) to 1 (death)], depending on the helminth species [11,12]. Other recognized disabilities included were massive dysentery syndrome for *T. trichiura* (DW 0.116), anaemia for hookworm and intestinal obstruction due to *A. lumbricoides* infections (each with a DW of 0.024). From these estimates it becomes evident that according to the current consensus, individuals infected with STHs usually do not experience overt morbidity or life-threatening manifestations. However, the chronic under-nourishment [13,14] and impaired physical and mental development [15] associated with such infections in childhood arguably prevent populations in which STH infections are pervasive to realize their full potential. Together with other unfavourable conditions, this threatens to perpetuate their entrapment in the vicious cycle of poverty and poor health [16].

Two decade ago, Stephenson and colleagues reported that the physical fitness, as determined by the Harvard step test (HST) [17], of Kenyan school boys infected with STHs had improved 7 weeks after the administration of a single dose of albendazole [18]. In another study by this group, which looked at physical fitness 4 months post-treatment, similar results were obtained [19]. Subsequently, little scientific inquiry was made, but recently, the question was re-visited in a cross-sectional survey carried out in Kenya. The

study confirmed that hookworm infection is negatively correlated with fitness scores in girls and teenage women aged 5-18 years. The reduced physical fitness was attributed to anaemia and stunting resulting from chronic hookworm infection [20]. However, a cross-sectional survey carried out in Côte d'Ivoire, with the aim of determining the effect of STHs and schistosome infections on physical fitness, failed to demonstrate a correlation in children aged 7-15 years [21]. Both studies employed the 20 m shuttle run test to measure fitness [22].

The objective of the present study was to contribute to the small evidence-base regarding the effect of helminth infections on physical fitness of school-aged children. The study was designed as a cross-sectional survey, assessing the technical and operational feasibility of measuring physical fitness among school-aged children in rural southwest P.R. China where STH infections are widespread.

8.3. Methods

8.3.1. Study sites

The study was carried out in the primary schools of three villages inhabited by members of the Bulang ethnic minority group, located in Menghai county, Xishuangbanna Dai autonomous prefecture, Yunnan province, P.R. China, from May to June 2011. The Bulang speak their own traditional language but the younger generation is increasingly learning to speak and write Mandarin Chinese. However, current literacy rates are still low, with most of the adults being illiterate or only having obtained primary education. The Bulang traditionally build their villages in mountainous regions, while the fertile plains are dominated by another minority, namely the Dai.

The area enjoys a subtropical climate characterized by heavy rainfall during the summer monsoon season and mild winter months. The Bulang rely on agriculture for income generation, with Pu'er tea as the most important cash crop. Livestock breeding is limited and pigs are the main domestic animals. General living standards are low. Systematic STH control activities have been implemented in the study villages since 2009 (periodic distribution of albendazole to the entire population, construction of family latrines in Manguo new village).

The three study villages are (i) Manguo new village (geographical coordinates: 21°45'09.01" N latitude and 100°18'47.20" E longitude); (ii) upper Nanwen

(21°46'02.15" N and 100°23'50.61" E); and (iii) lower Nanwen (21°46'34.02" N and 100°23'56.89" E), all located at high altitudes (1550-1650 m above sea level). These villages are comparable among themselves and to other Bulang villages in terms of socio-demography, general level of development, infrastructure and other features. Important characteristics of Bulang villages have been described in detail in previous publications pertaining to the epidemiology and control of STHs in Bulang communities [23-26].

8.3.2. Study design

The present study pursued a cross-sectional design. Participants submitted stool samples for subsequent parasitological examination in a laboratory using light microscopy. Anthropometric indicators (height and weight), haemoglobin and family-level socio-economic conditions were assessed. Children's physical fitness was determined based on their performance in a 20-m shuttle run test.

8.3.3. Ethical considerations

The study protocol was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland). Ethical clearance was granted by the ethics committee in Basel (EKBB, reference no. 144/11) and the Academic Board of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, P.R. China).

The village chiefs, doctors, teachers and study participants were briefed on the study aims and procedures. Children who were absent from school or suffering from any major illness were excluded from the study. All other pupils aged 8-15 years were invited to participate. Since most parents/guardians are illiterate, oral informed consent was sought. Children could withdraw from the study anytime without further obligations. Upon registration, each study participant was given a unique ID number. In all subsequent steps, this number was used to ensure that all results were kept anonymous. At the end of the study, sufficient albendazole to treat the entire communities was handed over to the village health authorities, who were in charge of distributing them to villagers aged 2 years and above (400 mg, single oral dose).

8.3.4. Study procedures

During registration, the age of the participant and the main source of income of his/her family were recorded. Participants were given a pre-labelled (name, ID) stool collection container and encouraged to submit one stool sample the following morning. Filled containers were collected between 07:00 and 09:00 hours and transported to the County Center for Disease Control and Prevention (CDC) laboratory in Menghai city for diagnostic work-up on the same day. Each stool sample was visually inspected for the presence of *Taenia* spp. proglottids. Subsequently, duplicate Kato-Katz thick smears were prepared from each sample, and read within 60 min after preparation, under 400x magnification [27]. Stratified by helminth species, the mean number of eggs from the duplicate thick smears was multiplied by a factor 24 to obtain an estimate of the number of eggs per gram (EPG) of stool [28]. For quality control, the two slides were independently read by different technicians and the results compared. Slides were re-read if inconsistencies were detected.

Field workers blinded to the infection status of the study participants performed the anthropometric measurements. Body weight was measured (to the nearest 0.1 kg) twice using a digital scale (Nantong Model RCS-150; Jiangsu, P.R. China), and averaged. For each measurement, the participant was asked to stand in the centre of the scale platform without shoes and sweaters. The height of each participant was also measured (to the nearest 0.1 cm) twice, and averaged. A stadiometer (Nantong Xineng Ltd, Jiangsu, P.R. China) was used and the participant stood, without shoes, with his/her back erect but shoulders relaxed against the stadiometer, while the headpiece was lowered to the crown of the head with sufficient pressure to compress the hair.

The body weight and height values were used to calculate the body mass index (BMI: defined as $\text{weight}/\text{height}^2$), BMI-for-age Z score (BAZ: an indicator for wasting) and height-for-age Z score (HAZ: an indicator for stunting) [29].

For haemoglobin measurements, a fresh set of alcohol swab, safety lancet and microcuvette was used for each participant. The ear lobe was pricked and the second drop of blood taken up by the microcuvette for reading with a HemoCue[®] Hb 301 system (HemoCue[®] AB; Ängelholm, Sweden). Anaemia was defined according to WHO age-specific cut-offs: Hb <11.5 g/dl for ages <12 years; Hb <12 g/dl for ages ≥12 years and <15 years; Hb <13 g/dl for males ≥15 years [30].

Lastly, a 20-m shuttle run test was performed. All participants were asked by the village doctor and teachers to indicate any body discomfort they might have, and the doctor stayed throughout the test to ensure that medical attention could be given immediately if required. The test was carried out between 10:00 and 12:00 hours in adherence to standard published procedures [20,22]. Participants, in groups of five, ran back and forth on a 20 m flat course, following the pace of pre-recorded sound signals (Team Bleep Test Version 1.3.1, Bitworks Design; Cheltenham, UK). Starting with a running speed of 8.5 km h⁻¹, the frequency of the signals indicating 20 m intervals increased by 0.5 km h⁻¹ every min. During the test, participants were encouraged constantly to complete as many courses as possible. When a participant failed to follow the pace for two consecutive 20 m intervals, he/she was asked to stop. The running speed from the last completed interval and the total number of laps completed were recorded. The age and speed were converted into the maximum volume of oxygen that can be utilized within 1 min during exhaustive exercise (VO₂ max estimate, expressed in ml kg⁻¹ min⁻¹) with the equation put forth by Léger et al. [22]. Physical fitness of each participant was expressed as the VO₂ max estimate and the number of laps completed.

8.3.5. Statistical analysis

Data were entered into Excel version 2008 (Microsoft Corp.; Redmond, WA, USA), double-checked and merged into a single database for statistical analysis with STATA version 10.0 (STATA Corp.; College Station, TX, USA). Children's parasitological status was assessed in terms of prevalence, infection intensity (mean EPG) and multiparasitism (concurrent infections with more than one helminth species). Anthropometric indicators, haemoglobin concentrations, VO₂ max estimate and the number of laps completed in the 20-m shuttle run test were expressed as means. Comparisons were made between participants infected with a particular STH species and participants not infected with that specific species. Test statistics included chi-square (χ^2), Fisher's exact, Wilcoxon rank-sum, Kruskal-Wallis, two sample *t*-tests and multiple linear regression models, as appropriate.

8.4. Results

8.4.1. Compliance and demography of study participants

All 79 children potentially available for the study received oral informed consent from their parents/guardians to participate in the study. All of them completed the 20-m shuttle run test and anthropometric and haemoglobin measurements. Sufficiently large stool samples for diagnostic work-up were provided by 69 children, resulting in an overall compliance of 87% (Figure 8.1). Subsequent analyses are based on this cohort of 69 children who had complete data records.

There were 40 girls (58%) and the median age of the study cohort was 11 years (range: 8-15 years). No significant difference was observed between the three villages with regard to the age distribution and sex ratio (both $P > 0.05$). All children were of Bulang ethnicity, and came from families with farming as their main source of income.

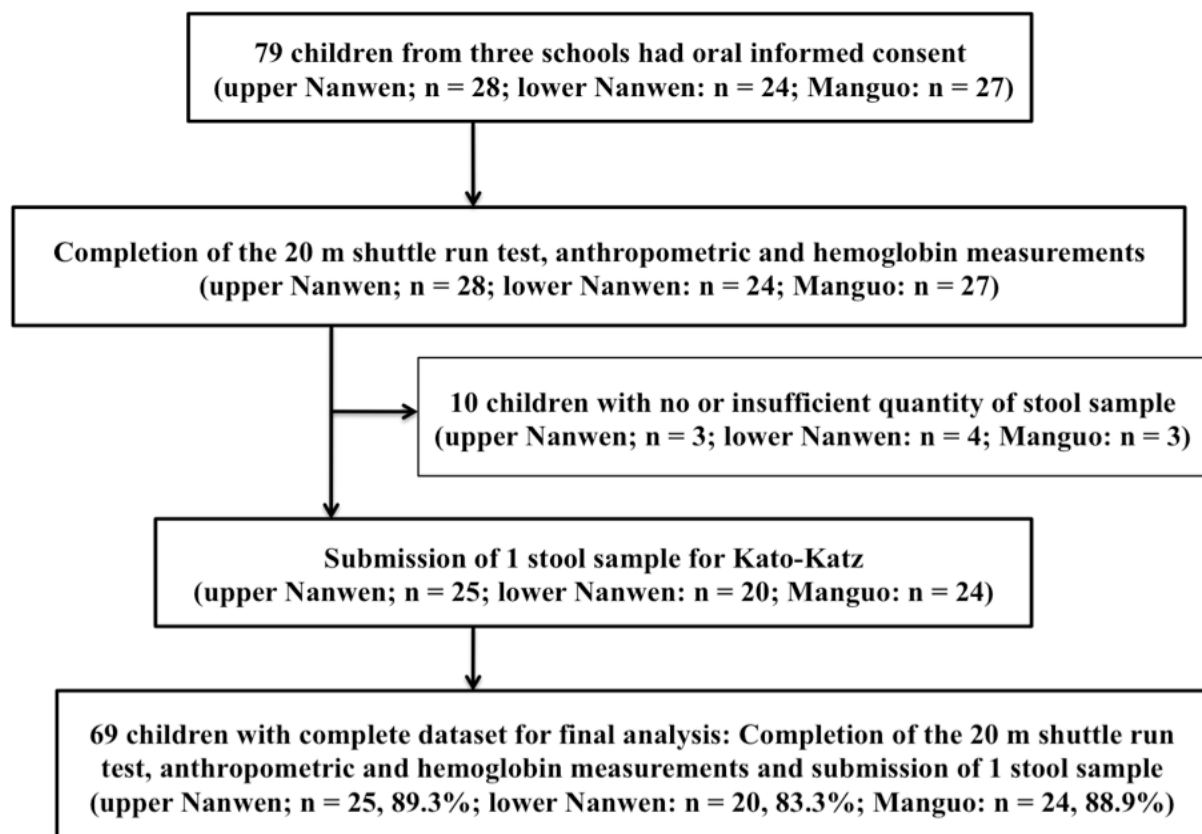


Figure 8.1. Study cohort and compliance of school-aged children from three Bulang villages, Yunnan province, P.R. China in mid-2011.

8.4.2. Soil-transmitted helminth infection status

Fifty-nine out of the 69 children (86%) were infected with at least one intestinal helminth species. Infection prevalence of *T. trichiura*, *A. lumbricoides* and hookworm were 81%, 44% and 6%, respectively. In addition, one infection with *Taenia* spp. was identified. Stratification by sex revealed that boys had higher mean EPG values than girls for all helminth species even though in terms of prevalence, fewer males were infected (Table 8.1). In addition, the odds of being infected with STHs decreased by 37% when age increased by a year. Furthermore, the prevalence of *T. trichiura* infection was significantly higher among younger children (8-11 years) compared to their older counterparts (12-15 years) ($P = 0.010$) (Table 8.1). Multiparasitism was common: slightly more than half of the infected children (51%) harboured dual-species infections (mostly *A. lumbricoides* plus *T. trichiura*). One triple-species infection was detected; the remaining 48% of the children were infected by only one species.

8.4.3. Anthropometric indices and haemoglobin in relation to parasitological status

The mean height, weight and BMI of the study cohort were 134.7 cm, 31.2 kg and 16.9 kg m⁻², respectively. As shown in Table 8.2, children infected with *T. trichiura* had significantly lower body weight, height and BMI than their peers who were *T. trichiura*-free. Stunting was observed in 59% of the participants, and 5.8% of them were wasted. No statistically significant associations between these two indicators and STH infection status were observed. The mean haemoglobin level was 15.7 g dl⁻¹ with no anaemia observed regardless of children's STH infection status.

8.4.4. Physical fitness in relation to parasitological status

The mean VO₂ max estimates of boys infected with *A. lumbricoides*, *T. trichiura* and hookworm were 44.5, 45.1 and 44.4 ml kg⁻¹ min⁻¹, respectively. A considerably higher mean VO₂ max estimate was determined for helminth-free boys (48.2 ml kg⁻¹ min⁻¹). For girls, no such differences were observed. However, the two girls infected with hookworm or *Taenia* spp. had lower VO₂ max estimates (37.8 and 40.3 ml kg⁻¹ min⁻¹, respectively) than helminth-free girls (41.8 ml kg⁻¹ min⁻¹). After stratification by age group, children infected with *A. lumbricoides* and *T. trichiura* had reduced mean VO₂ max estimates compared to their peers in the same age class who were not infected with either parasite. A statistically significant difference ($P = 0.027$) was observed between

11- to 12-year-old children infected with *A. lumbricoides* ($42.0 \text{ ml kg}^{-1} \text{ min}^{-1}$) and their counterparts not infected with this parasite ($44.2 \text{ ml kg}^{-1} \text{ min}^{-1}$; Table 8.3).

In terms of number of 20-m laps successfully completed by the participants, both boys and girls infected with *A. lumbricoides* and *T. trichiura* achieved less laps than their non-infected counterparts (Table 8.4). This difference reached statistical significance in boys with or without *A. lumbricoides* infection (27.4 laps *versus* 36.1 laps, $P = 0.045$). Similarly, boys infected with *T. trichiura* completed 30.5 laps while their counterparts not infected with this parasite completed significantly more laps (44.5 laps, $P = 0.003$). Stratified by age group, significant differences were observed among children aged 11-12 years depending on their infection status with *A. lumbricoides* (21.3 laps among infected and 28.7 laps among non-infected children, $P = 0.019$), and among children aged 8-10 years and their *T. trichiura* infection status (23.9 laps among infected and 35.3 laps among non-infected, $P = 0.028$).

According to the multiple linear regression models presented in Table 8.5, sex, stunting and *T. trichiura* infection showed significant negative correlations with mean VO_2 max estimates and number of 20-m laps completed. *T. trichiura* infection in children lowered the mean VO_2 max estimate by $1.94 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P = 0.005$) and resulted in 6.1 fewer laps compared to children without *T. trichiura* infection ($P = 0.004$). In addition, the mean VO_2 max estimate of stunted children was lowered by $1.63 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P = 0.002$) and they completed 5.3 laps less compared to children of standard stature (P -value = 0.001).

Table 8.1. Prevalence and intensity of soil-transmitted helminth infection (mean of duplicate Kato-Katz thick smears) in 69 school-aged Bulang children from Yunnan province, P.R. China, stratified by sex and age group.

		Sex			Age (years)			
		Male (N ^a = 29)	Female (N ^a = 40)	P ^c	8-10 (N ^a = 22)	11-12 (N ^a = 33)	13-15 (N ^a = 14)	P ^c
		n ^b (%)	n ^b (%)		n ^b (%)	n ^b (%)	n ^b (%)	
<i>T. trichiura</i>	Overall prevalence: 81%							
	Prevalence	23 (79)	33 (83)	0.738	19 (86)	29 (88)	8 (57)	0.036
	Infection intensity^d							
	Mean EPG; SE ^e	419; 582	350; 506	0.565	486; 643	374; 515	140; 162	0.194
	Light (1-999)	20 (69)	29 (73)		16 (73)	25 (76)	8 (57)	
	Moderate (1000-9999)	3 (10)	4 (10)		3 (14)	4 (12)	n.r.	
<i>A. lumbricoides</i>	Overall prevalence: 44%							
	Prevalence	9 (31)	21 (53)	0.076	13 (59)	12 (36)	5 (36)	0.201
	Infection intensity^d							
	Mean EPG; SE ^e	30,719; 60,310	12,783; 29,677	0.701	23,557; 42,666	16,694; 48,090	7670; 11,1856	0.331
	Light (1-4999)	6 (21)	13 (33)		7 (32)	9 (27)	3 (21)	
	Moderate (5000-49,999)	1 (3.5)	7 (18)		4 (18)	2 (6.1)	2 (14)	
	Heavy (≥50,000)	2 (6.9)	1 (2.5)		2 (9.1)	1 (3.0)	n.r.	
Hookworm	Overall prevalence: 6%							
	Prevalence	3 (10)	1 (2.5)	0.302	n.r.	3 (9.1)	1 (7.1)	0.409
	Infection intensity^d							
	Mean EPG; SE ^e	56; 66	12; n.a.	0.346	n.r.	56; 66	12; n.a.	0.371
	Light (1-1999)	3 (10)	1 (2.5)			3 (9.1)	1 (7.1)	
<i>Taenia</i> spp.	Prevalence	n.r.	1 (2.5)	1.000	n.r.	1 (3.0)	n.r.	1.000

^aN: total sample size; ^bn: number of infected individuals; ^cAll *P*-values are calculated using χ^2 , Fisher's exact, Wilcoxon rank-sum or Kruskal-Wallis test, as appropriate; ^dStratified according to WHO guidelines; ^eArithmetic mean among the infected; standard error (SE); n.a., not applicable; n.r.: not represented

Table 8.2. Anthropometric indicators and haemoglobin concentration, in relation to parasitological status, of 69 school-aged Bulang children from Yunnan province, P.R. China in mid-2011.

	<i>T. trichiura</i>			<i>A. lumbricoides</i>			Hookworm		
	Non-infected (n = 13)	Infected (n = 56)	<i>P</i> ^a	Non-infected (n = 39)	Infected (n = 30)	<i>P</i> ^a	Non-infected (n = 65)	Infected (n = 4)	<i>P</i> ^a
Anthropometric									
Mean weight [kg] (range)	36.0 (21.3-47.9)	30.1 (16.8-53.7)	0.020	31.9 (18.1-53.7)	30.3 (16.8-47.9)	0.433	30.9 (16.8-53.7)	37.2 (29.4-42.9)	0.138
Mean height [cm] (range)	140.5 (118.0-154.9)	133.3 (110.3-155.9)	0.037	136.6 (116.5-155.9)	132.2 (110.3-151.1)	0.111	134.2 (110.3-155.9)	142.8 (134.0-151.5)	0.147
Mean BMI [kg m ⁻²] (range)	18.0 (15.3-21.8)	16.6 (13.3-23.5)	0.034	16.8 (13.3-23.5)	17.0 (13.8-21.5)	0.729	16.8 (13.3-23.5)	18.2 (16.3-21.5)	0.212
% wasted ^b	n.r.	7.1	1.000	10.3	n.r.	0.127	6.5	n.r.	1.000
% stunted ^c	53.9	60.7	0.650	56.4	63.3	0.562	60.0	50.0	0.693
Haematologic									
Mean haemoglobin [g dl ⁻¹] (range)	14.1 (11.6-17.5)	16.1 (12.5-20.1)	<0.001	15.3 (11.6-18.9)	16.2 (12.5-20.1)	0.043	13.4 (11.0-15.6)	14.3 (13.4-15.2)	0.147

^aAll *P*-values are calculated using two-sample t test, Wilcoxon rank-sum, χ^2 or Fisher's exact test, as appropriate^b Wasting is defined as ≤ -2 in BAZ score^c Stunting is defined as ≤ -2 HAZ score

n.r., not represented

Table 8.3. Mean VO₂ max estimates^b (ml kg⁻¹ min⁻¹), in relation to parasitological status, of 69 Bulang primary school children from Yunnan province, P.R. China in mid-2011, stratified by sex and age group.

	<i>T. trichiura</i>			<i>A. lumbricoides</i>			Hookworm		
	Non-infected (n = 13)	Infected (n = 56)	<i>P</i> ^a	Non-infected (n = 39)	Infected (n = 30)	<i>P</i> ^a	Non-infected (n = 65)	Infected (n = 4)	<i>P</i> ^a
Sex									
Male (n = 29)	47.7 (44.1-51.3)	45.1 (43.7-46.5)	0.097	46.1 (44.5-47.8)	44.5 (42.2-46.9)	0.242	45.8 (44.4-47.2)	44.4 (34.9-53.9)	0.504
Female (n = 40)	41.9 (40.5-44.3)	43.3 (42.2-44.3)	0.263	42.7 (41.3-44.2)	43.3 (42.1-44.6)	0.513	43.2 (42.3-44.1)	37.8 (n.a.)	n.a
Age (years)									
8-10 (n = 22)	48.7 (36.8-60.5)	46.5 (45.5-47.5)	0.171	47.8 (45.3-50.3)	46.1 (45.1-47.2)	0.136	46.8 (45.7-47.9)	n.r.	n.a.
11-12 (n = 33)	43.5 (41.5-45.5)	43.4 (42.3-44.5)	0.935	44.2 (42.9-45.4)	42.0 (40.9-43.2)	0.027	43.5 (42.6-44.4)	42.0 (28.9-55.1)	0.358
13-15 (n = 14)	43.3 (39.5-47.1)	40.4 (38.5-42.4)	0.100	41.9 (39.1-44.8)	41.2 (38.5-43.9)	0.701	41.4 (39.5-43.3)	44.0 (n.a.)	n.a.

^a All *P*-values calculated using a two-sample *t*-test^b All mean VO₂ estimates are expressed in ml kg⁻¹ min⁻¹, with 95% confidence intervals in brackets when appropriate

n.a., not applicable; n.r., not represented

Table 8.4. Mean number of 20-m laps^b completed by 69 school-aged Bulang children from Yunnan province, P.R. China in mid-2011, in relation to parasitological status, stratified by sex and age group.

	<i>T. trichiura</i>			<i>A. lumbricoides</i>			Hookworm		
	Non-infected (n = 13)	Infected (n = 56)	<i>P</i> ^a	Non-infected (n = 39)	Infected (n = 30)	<i>P</i> ^a	Non-infected (n = 65)	Infected (n = 4)	<i>P</i> ^a
Sex									
Male (n = 29)	44.5 (35.8-53.2)	30.5 (26.4-34.6)	0.003	36.1 (31.1-41.0)	27.4 (20.4-34.5)	0.045	33.0 (28.7-37.3)	36.7 (29.3-37.5)	0.587
Female (n = 40)	26.0 (20.7-31.3)	22.3 (20.7-23.9)	0.063	24.4 (21.9-26.8)	21.7 (19.7-23.6)	0.076	23.3 (21.8-24.7)	12.0 (n.a.)	n.a.
Age (years)									
8–10 (n = 22)	35.3 (0-73.8)	23.9 (21.7-29.3)	0.028	29.6 (21.6-37.6)	22.7 (19.2-26.2)	0.061	25.5 (21.7-29.3)	n.r.	n.a.
11–12 (n = 33)	28.3 (17.6-38.9)	25.7 (22.2-29.2)	0.595	28.7 (24.3-33.0)	21.3 (18.3-24.4)	0.019	25.9 (23.0-28.8)	27.0 (0-73.5)	0.841
13–15 (n = 14)	38.3 (25.2-51.5)	29.6 (24.2-35.1)	0.115	35.1 (26.7-43.5)	30.2 (19.3-41.1)	0.408	32.8 (26.5-39.0)	41.0 (n.a.)	n.a.

^a All *P*-values calculated using a two-sample *t*-test^b Mean number of laps completed with 95% confidence intervals in brackets when appropriate

n.a., not applicable; n.r., not represented

Table 8.5. Multiple linear regression models with mean VO₂ max estimates (ml kg⁻¹ min⁻¹) (I) or number of 20-m laps completed (II) as outcomes and age, sex, stunting and infection status as explanatory variables. Data is derived from 69 Bulang primary school children from Yunnan province, P.R. China, in mid-2011.

(I)			
Multiple linear regression^a			
Explanatory variables	Coefficient	95% confidence interval	P
Age (in years)	-1.37	-1.67 to -1.08	<0.001
Sex (reference: male)	-2.36	-3.38 to -1.35	<0.001
Stunting (reference: not stunted)	-1.63	-2.63 to -0.63	0.002
<i>A. lumbricoides</i> (reference: not infected)	-0.98	-2.03 to 0.07	0.066
<i>T. trichiura</i> (reference: not infected)	-1.94	-3.26 to -0.62	0.005

^a Only explanatory variables that have showed significant difference in the descriptive statistics were included.

Key indicators of model: F (5, 63) = 23.97; $p < 0.001$; R-squared = 0.66

(II)			
Multiple linear regression^b			
Explanatory variables	Coefficient	95% confidence interval	P-value
Age (in years)	0.86	-0.07 to 1.79	0.069
Sex (reference: male)	-9.89	-13.08 to -6.70	<0.001
Stunting (reference: not stunted)	-5.32	-8.48 to -2.17	0.001
<i>A. lumbricoides</i> (reference: not infected)	-2.83	-6.12 to 0.46	0.090
<i>T. trichiura</i> (reference: not infected)	-6.14	-10.29 to -1.99	0.004

^b Only explanatory variables that have showed significant difference in the descriptive statistics were included.

Key indicators of model: F (5, 63) = 16.94; $p < 0.001$; R-squared = 0.57

8.5. Discussion

In this cohort of school-aged children belonging to the Bulang ethnic minority in Yunnan province, southwest P.R. China, we found significantly impaired physical fitness due to STH infections. Indeed, children who were infected with *T. trichiura* or stunted had significantly lower mean VO_2 max estimates and completed significantly fewer 20-m laps in a shuttle run test than children without *T. trichiura* infection and who were of standard stature. An earlier study also conducted in a Bulang community located in close proximity to the current setting revealed prevalences for each of the three STHs above 85% with almost two-third (62.3%) of the participants harbouring three helminth species concurrently [23]. We found somewhat lower prevalences, most likely as a result of recent STH control efforts. Moreover, in the previous investigation a suite of diagnostic methods was employed, which increased the diagnostic sensitivity. The high prevalence of stunting, regardless of the present STH infection status, might indicate that virtually all children have experienced STH infections at some point in their life. This is supported by the high prevalences reported previously, and would mean that it is difficult to draw conclusions from comparisons of long-term growth indicators with current infection status as it is done in a cross-sectional study design. Longitudinal monitoring would be required instead. In terms of infection intensities and multiparasitism, no clear relationship was observed between infection intensity and physical fitness, but an increase in physical fitness impairment was observed in children with dual or triple species infection as compared to children with single species infections. Due to the small overall sample size, very small groups resulted after stratification by species-specific infection intensity and multiparasitism. Therefore, results should not be over-interpreted.

Assessments of physical fitness in relation to STH infection status have mainly relied on the HST [17] and the 20-m shuttle run test [22]. During pilot-testing, we found that it was difficult to standardize the HST across the different villages and that children took a longer time to learn and perform this test properly as compared to the 20-m shuttle run test. Thus, the 20-m shuttle run test was chosen for the current study. In the 20-m shuttle run test, the particular pace at which the signals are sounded for a min is also termed as a stage and within a stage, there are several sub-stages to complete for that pace. When estimating the mean VO_2 max, using the equation put forth by Léger et al. [22], these sub-stages are not taken into account. We argue that sub-stages might not be of significance in healthy children of normal growth but given that the burden due to STH

infections in children is still ambiguous, it might be important to consider such subtleties when studying the difference in physical fitness between infected and non-infected children. Hence, the number of completed 20-m laps was used as an additional outcome measure on top of the mean VO_2 max estimate. We speculate that this simple indicator could serve as a straightforward measure of physical fitness [32].

There are some limitations to this study. First, our study was designed as a cross-sectional survey and as such could only identify associations rather than causality. Second, the overall sample size was small. Third, only a single stool sample was collected from each participant. Hence, some STH infections, particularly those of light intensity, were probably missed, as seen in other studies where multiple stool samples and a combination of diagnostic methods had been employed [23,32,33].

Despite these limitations, as a proof-of-concept, our study has shown the feasibility of conducting physical fitness testing along with stool examination, anthropometric measurements and determining haemoglobin levels in an ethnic minority group of P.R. China. Hence, our study confirms previous experiences in different African settings, where school-aged children were also receptive to physical fitness tests to determine whether physical fitness was negatively impacted by helminth infections [21,31].

Most Bulang families are engaged in agriculture. It is therefore conceivable that reduced physical fitness translates into lowered work productivity or increased exhaustion. The high prevalence of STHs and the marked differences in physical fitness and anthropometric measures between infected and non-infected children, which in certain cases reached statistical significance even in our small sample, are of considerable concern. These preliminary findings warrant larger follow-up studies. We have launched a new study with a larger cohort of 9- to 12-year-old Bulang children. In a baseline survey, children are rigorously diagnosed for STH infections, followed by random allocation of infected children into a treatment group (triple dose albendazole) or a placebo group, and monitoring of physical fitness over a 7-month period post-treatment.

8.6. Conclusions

In summary, our study provided a snapshot of the effect of STH infections on the growth and physical fitness of school-aged children in an ethnic minority group of rural southwest P.R. China. Our preliminary results suggest that children who were stunted or

infected with *T. trichiura* had reduced physical fitness. The current study confirmed the feasibility of implementing physical fitness tests in a rural, resource-constraint setting, and provided the basis for a more elaborate study, currently ongoing, to investigate the effect of de-worming on physical fitness in school-aged children.

8.7. Conflicting interests

The authors declared that they have no financial, professional or personal conflicting interests related to this article.

8.8. Authors' contributions

PY designed and implemented the study, entered, analyzed and interpreted the data and prepared the manuscript; ZWD, RC, LPZ, FWW, JW, XZW, HZ assisted in the design and implementation of study; XNZ designed the study, supervised its implementation and revised the manuscript; JU designed the study, interpreted the data and revised the manuscript; PS designed the study, facilitated its implementation, supervised PY, interpreted the data and revised the manuscript. All authors read and approved the final manuscript prior to submission.

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8.10. References

1. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ: **Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm.** *Lancet* 2006, **367**:1521-1532.
2. Brooker S, Clements ACA, Bundy DAP: **Global epidemiology, ecology and control of soil-transmitted helminth infections.** *Adv Parasitol* 2006, **62**:221-261.
3. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Erlich Sachs S, Sachs JD, Savioli L: **Control of neglected tropical diseases.** *N Engl J Med* 2007, **357**:1018-1027.
4. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J: **Helminth infections: the great neglected tropical diseases.** *J Clin Invest* 2008, **118**:1311-1321.
5. Brooker S: **Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers - a review.** *Int J Parasitol* 2010, **40**:1137-1144.
6. Utzinger J, Raso G, Brooker S, De Savigny D, Tanner M, Ornberg N, Singer BH, N'Goran E K: **Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution.** *Parasitology* 2009, **136**:1859-1874.
7. Albonico M, Montresor A, Crompton DWT, Savioli L: **Intervention for the control of soil-transmitted helminthiasis in the community.** *Adv Parasitol* 2006, **61**:311-348.
8. WHO: **Preventive chemotherapy in human helminthiasis: coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme managers.** Geneva: World Health Organization; 2006.
9. Steinmann P, Utzinger J, Du ZW, Zhou XN: **Multiparasitism: a neglected reality on global, regional and local scale.** *Adv Parasitol* 2010, **73**:21-50.
10. Ezeamama AE, Friedman JF, Olveda RM, Acosta LP, Kurtis JD, Mor V, McGarvey ST: **Functional significance of low-intensity polyparasite helminth infections in anemia.** *J Infect Dis* 2005, **192**:2160-2170.
11. Mathers CD, Ezzati M, Lopez AD: **Measuring the burden of neglected tropical diseases: the global burden of disease framework.** *PLoS Negl Trop Dis* 2007, **1**:e114.
12. WHO: **The global burden of disease: 2004 update.** Geneva: World Health Organization; 2008.

13. Mupfasoni D, Karibushi B, Koukounari A, Ruberanziza E, Kaberuka T, Kramer MH, Mukabayire O, Kabera M, Nizeyimana V, Deville MA, et al: **Polyparasite helminth infections and their association to anaemia and undernutrition in northern Rwanda.** *PLoS Negl Top Dis* 2009, **3**:e517.
14. Stephenson LS, Latham MC, Ottesen EA: **Malnutrition and parasitic helminth infections.** *Parasitology* 2000, **121** Suppl:S23-38.
15. Eppig C, Fincher CL, Thornhill R: **Parasite prevalence and the worldwide distribution of cognitive ability.** *Proc Biol Sci* 2010, **277**:3801-3808.
16. Sachs JD: **The MDG decade: looking back and conditional optimism for 2015.** *Lancet* 2010, **376**:950-951.
17. Gallagher JR, Brouha L: **A simple method of testing the physical fitness of boys.** *Am Assn Health Phys Ed Rec Res Q* 1943, **14**:23-31.
18. Stephenson LS, Latham MC, Kinoti SN, Kurz KM, Brigham H: **Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole.** *Trans R Soc Trop Med Hyg* 1990, **84**:277-282.
19. Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A: **Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole.** *J Nutr* 1993, **123**:1036-1046.
20. Bustinduy AL, Thomas CL, Fiutem JJ, Parraga IM, Mungai PL, Muchiri EM, Mutuku F, Kitron U, King CH: **Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric.** *PLoS Negl Top Dis* 2011, **5**:e1213.
21. Müller I, Coulibaly JT, Fürst T, Knopp S, Hattendorf J, Krauth SJ, Stete K, Righetti AA, Glinz D, Yao AK, Pühse U, N'Goran EK, Utzinger J: **Effect of schistosomiasis and soil-transmitted helminth infections on physical fitness of school children in Côte d'Ivoire.** *PLoS Negl Top Dis* 2011, **5**:e1239.
22. Léger LA, Mercier D, Gadoury C, Lambert J: **The multistage 20 metre shuttle run test for aerobic fitness.** *J Sports Sci* 1988, **6**:93-101.
23. Steinmann P, Du ZW, Wang LB, Wang XZ, Jiang JY, Li LH, Marti H, Zhou XN, Utzinger J: **Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods.** *Am J Trop Med Hyg* 2008, **78**:760-769.
24. Steinmann P, Utzinger J, Du ZW, Jiang JY, Chen JX, Hattendorf J, Zhou H, Zhou XN: **Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp.: a randomized controlled trial.** *PLoS One* 2011, **6**:e25003.

25. Steinmann P, Zhou XN, Du ZW, Jiang JY, Xiao SH, Wu ZX, Zhou H, Utzinger J: **Tribendimidine and albendazole for treating soil-transmitted helminths, *Strongyloides stercoralis* and *Taenia* spp.: open-label randomized trial.** *PLoS Negl Trop Dis* 2008, **2**:e322.
26. Ziegelbauer K, Steinmann P, Zhou H, Du ZW, Jiang JY, Furst T, Jia TW, Zhou XN, Utzinger J: **Self-rated quality of life and school performance in relation to helminth infections: case study from Yunnan, People's Republic of China.** *Parasit Vectors* 2010, **3**:61.
27. Katz N, Chaves A, Pellegrino J: **A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni.** *Rev Inst Med Trop São Paulo* 1972, **14**:397-400.
28. WHO: **Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee.** *WHO Tech Rep Ser* 2002, **912**:i-vi, 1-57, back cover.
29. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J: **Development of a WHO growth reference for school-aged children and adolescents.** *Bull World Health Organ* 2007, **85**:660-667.
30. WHO: **Assessing the iron status of populations: report of a Joint World Health Organization/Centers for Disease Control and Prevention. Technical consultation on the assessment of iron status at the population level.**, 2nd edition. Geneva: World Health Organization; 2007.
31. Bustinduy AL, Thomas CL, Fiutem JJ, Parraga IM, Mungai PL, Muchiri EM, Mutuku F, Kitron U, King CH: **Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric.** *PLoS Negl Trop Dis* 2011, **5**:e1213.
32. Kriemler S, Zahner L, Schindler C, Meyer U, Hartmann T, Hebestreit H, Brunner-La Rocca HP, van Mechelen W, Puder JJ: **Effect of school based physical activity programme (KISS) on fitness and adiposity in primary schoolchildren: cluster randomised controlled trial.** *BMJ* 2010, **340**:c785.
33. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, Marti H, Utzinger J: **Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques.** *PLoS Negl Trop Dis* 2008, **2**:e331.
34. Booth M, Vounatsou P, N'Goran E K, Tanner M, Utzinger J: **The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing *Schistosoma mansoni* and hookworm co-infections in rural Cote d'Ivoire.** *Parasitology* 2003, **127**:525-531.

9. Effect of deworming on physical fitness of school-aged children in Yunnan, China: a double-blind, randomized, placebo-controlled trial

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9.1. Abstract

Background: There is considerable debate on the health impacts of soil-transmitted helminth infection. We assessed the effect of deworming on physical fitness and strength of children in an area highly endemic for soil-transmitted helminthiasis in Yunnan, People's Republic of China.

Methodology: A double-blind, randomized, placebo-controlled trial was conducted between October 2011 and May 2012. Children, aged 9-12 years, were treated with either triple-dose albendazole or placebo, and monitored for 6 months posttreatment. The Kato-Katz and Baermann techniques were used for the diagnosis of soil-transmitted helminths. Physical fitness was assessed with a 20-m shuttle run test, where the maximum aerobic capacity within 1 min of exhaustive exercise (VO_2 max estimate) and number of 20-m laps completed were recorded. Physical strength was determined with grip strength and standing broad jump tests. Body height and weight, sum of skinfolds, and hemoglobin level were recorded as secondary outcomes.

Principal Findings: Children receiving triple-dose albendazole scored slightly higher values in the primary and secondary outcomes than placebo recipients, but the difference lacked statistical significance. The increase in VO_2 max estimate from baseline was $1.6 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P=0.02$) less and the increase in the number of 20-m laps completed from baseline was five 20-m laps ($P=0.04$) less for *T. trichiura*-infected children compared to their non-infected peers. Similar trends were detected in the VO_2 max estimate and grip strength of children infected with hookworm and *Ascaris lumbricoides*, respectively. In addition, children with low infection intensity of *T. trichiura* and hookworm had consistently more increase in the VO_2 max estimate from baseline than their peers with high infection intensity of all soil-transmitted helminths (range: $1.9\text{-}2.1 \text{ ml kg}^{-1} \text{ min}^{-1}$; all $P < 0.05$).

Conclusions/Significance: We found no strong evidence for significant improvements in physical fitness and anthropometric indicators due to deworming. However, the negative effect of *T. trichiura* infection on physical fitness warrants further investigation.

Keywords: soil-transmitted helminths, Kato-Katz technique, Baermann technique, physical fitness, physical strength, anthropometry

9.2. Author summary

Children from the developing world are often burdened with intestinal worms due to poor water supply, sanitation and hygiene. However, the assessment of the burden of intestinal worms is difficult, and thus, the benefit of deworming is unclear. In this study, we determined the effect of deworming on the physical fitness and strength of 9- to 12-year-old children in Yunnan, China, where intestinal worms are common. Children were treated with triple-dose albendazole or placebo and monitored over a 6-month period. Stool samples were collected for the diagnosis of worm infections. Physical fitness was estimated with a 20-m shuttle run test and physical strength was assessed with grip strength and standing broad jump tests. Children receiving triple-dose albendazole scored slightly higher values in the primary and secondary outcomes than those children who were given placebo. However, the differences were not significant. We also found that children infected with intestinal worms performed significantly worse in a battery of physical fitness and strength tests than their non-infected counterparts. In particular, the negative impact of whipworm infection on physical fitness warrants further investigation.

9.3. Introduction

Soil-transmitted helminths, namely *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*), are the most common parasitic worm infections among humans. Indeed, more than 1 billion people are infected and approximately 5.4 billion people at risk [1-3]. In 2011, an estimated 875 million children, 70% of whom are school-aged, were at risk globally [4]. Impoverished communities with poor hygiene and lack of access to clean water and improved sanitation are especially vulnerable [5,6].

The global burden of soil-transmitted helminthiases is currently estimated at 5.2 million disability-adjusted life years (DALYs), mainly due to sub-clinical morbidities, but also including anemia and reduced cognitive and physical development [7-9]. Infections are largely chronic and usually asymptomatic, and hence the study and quantification of morbidity associated with soil-transmitted helminth infections are difficult, and only few studies have ventured to do so. In particular, no conclusive evidence has yet been established whether reduced physical fitness or strength can be a consequence of soil-transmitted helminth infections. Physical fitness has been positively correlated with academic performance through enhanced memory and attention [10,11], while physical strength is demanded in labor-intensive agriculture jobs, which often provide the main source of income in rural communities of the developing world [12]. A lack in both attributes due to soil-transmitted helminthiasis could arguably prevent school-aged children living in impoverished conditions from realizing their full potential and perpetuate their entrapment in the vicious cycle of poverty and poor health [13,14].

Based on the rationale that lowering infection intensity would help to control morbidity associated with chronic helminth infection, and that morbidity is infection intensity-dependent, the World Health Organization (WHO) advocates periodic deworming of at-risk populations (e.g., school-aged children and pregnant women) with single-dose albendazole (400 mg) or mebendazole (500 mg) [15,16]. Such an approach indeed reduces infection intensity in the target population, but high-quality evidence on the health benefits of de-worming in children is scant [17,18]. Two randomized controlled trials have shown that physical fitness in school boys infected with soil-transmitted helminths improved 7 weeks to 4 months after treatment with

single-dose albendazole [19,20]. In addition, although physical fitness was negatively correlated with *T. trichiura* and hookworm infections in two cross-sectional surveys [21,22], another cross-sectional survey did not find any correlation between physical fitness and soil-transmitted helminth infections [23]. However, it is important to note that in the latter study, both the prevalence and intensity of soil-transmitted helminth infections were very low.

We designed a randomized controlled trial to investigate the health benefits of deworming and thereby deepen our understanding of the burden caused by soil-transmitted helminth infection among school-aged children in a highly endemic area in the People's Republic of China (P.R. China). We assessed the effects of triple-dose albendazole on physical fitness and strength of initially soil-transmitted helminth-infected children, and studied the dynamics over a 6-month period posttreatment. Changes in anthropometric indicators and hemoglobin levels were also measured, and are reported as secondary outcomes.

9.4. Methods

9.4.1. Ethics statement

The study protocol was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland). The ethics committee of Basel (EKBB, reference no. 144/11) and the Academic Board of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, P.R. China) provided ethical clearance. The trial is registered with Current Controlled Trials (identifier: ISRCTN 25371788).

The village doctor, chief, and teachers of each village were briefed on the aims of the study. With help from the teachers, the investigators further explained the procedures to the children and their parents/guardians. Written informed consent was obtained from parents/guardians, whereas children assented orally. Data were kept anonymous. After the 6-month final follow-up, all children attending the five schools were given triple-dose albendazole (3 x 400 mg) irrespective of their infection status, study participation, and treatment during the study. Children diagnosed with *Strongyloides stercoralis* were given a single dose of ivermectin (200 µg/kg).

9.4.2. Participants

Participants were recruited from five primary schools, where a 70% or higher prevalence of soil-transmitted helminth infections had been detected during a rapid appraisal. All schools belong to villages exclusively inhabited by the Bulang ethnic minority group, and are located in the mountainous Bulangshan township bordering Myanmar, a sub-division of Menghai county in Xishuangbanna Dai autonomous prefecture, situated in Yunnan province, P.R. China. The five villages are: (i) Sandui (geographical coordinates: 21°33'07'' N latitude, 100°19'34'' E longitude, altitude: 1,566 m above sea level (asl)); (ii) Kongkan (21°32'34'' N, 100°20'25'' E, 1,195 m asl); (iii) Laozhai (21°31'37'' N, 100°18'01'' E, 1,399 m asl); (iv) Laonandong (21°33'28'' N, 100°21'45'' E, 1,188 m asl); and (v) Mannuo (21°33'27'' N, 100°23'53'' E, 1,352 m asl). Prior to the current trial, no survey or control activities targeting soil-transmitted helminthiasis have been implemented in the study villages. Detailed information on the study area has been published along with data on soil-transmitted helminth re-infection patterns among participants [24]. Moreover, the epidemiology and control of soil-transmitted helminthiasis in comparable Bulang communities previously studied by our group have been described elsewhere [25,26].

9.4.3. Study design

The study was designed as a double-blind, randomized, placebo-controlled trial with three follow-ups, and was carried out between October 2011 and May 2012. Assuming a prevalence of 70% with any soil-transmitted helminth infection and 50% loss to follow-up, the trial aimed to enroll 250 children at baseline to achieve a power of 80% at an alpha error of 5% for the detection of a $2.5 \text{ ml kg}^{-1} \text{ min}^{-1}$ difference in the maximum aerobic capacity within 1 min of exhaustive exercise ($\text{VO}_2 \text{ max}$ estimate) between the intervention and placebo groups.

Inclusion criteria for the trial were: (i) provision of two stool samples at baseline; (ii) presence of at least one type of soil-transmitted helminth infection; (iii) no deworming treatment within 6 months before the current study; (iv) no known allergy to albendazole; (v) no major systemic illnesses as determined by a medical doctor; (vi) no concurrent participation in other clinical trials; and (vii) residency in the study area for at least 1 year before enrollment.

Children aged 9-12 years who met the inclusion criteria were enrolled by field investigators for a baseline assessment involving parasitological examination, physical fitness and strength tests, and anthropometric and hemoglobin measurements. The same measurements were repeated 1, 4, and 6 months after treatment, with the exception of anthropometric indicators that were only re-assessed at the 4- and 6-month follow-ups (Figure 8.1).

The treatment allocation sequence was generated by a statistician using block randomization with randomly varying block sizes of 2, 4, and 6. Albendazole and placebo tablets were packaged by staff not involved in the field work into sealed envelopes marked with unique identifiers. Following the order of the class list provided by the teachers, each child was sequentially assigned a random number, which corresponds to a number on the sealed envelope. Both children and field investigators were blinded to the nature of the tablets. The assigned triple-dose treatment (i.e., 3 x 400 mg albendazole (GlaxoSmithKline; London, United Kingdom) or 3 x shape- and color-matched placebo (Fagron; Barsbüttel, Germany)), was started on treatment day 1 with a single dose, with subsequent doses administered every day until treatment day 3.

9.4.4. Field and laboratory procedures

Two stool samples were collected from each child on consecutive days. Both the Kato-Katz (duplicate slides per sample) and Baermann techniques (one examination per sample) were used; Kato-Katz for the detection of eggs of *A. lumbricoides*, hookworm, and *T. trichiura*, and Baermann for larvae of *S. stercoralis* [27]. Additionally, stool samples were visually inspected for *Taenia* spp. proglottids. For quality control, the duplicate Kato-Katz slides were examined independently and results compared. Slides were re-read if inconsistencies were detected.

Physical fitness was estimated with the 20-m shuttle run test [22]. The running speed from the last completed 20-m lap and the total number of intervals completed were recorded. The child's age and speed were then converted into VO_2 max estimate (to the nearest $0.1 \text{ ml kg}^{-1} \text{ min}^{-1}$) with an equation put forth by Léger *et al.* [28].

Physical strength was assessed with the grip strength and standing broad jump tests. For the grip strength test, the hand span (distance from the tip of the thumb to

the tip of the little finger) of the child's dominant hand was measured (to the nearest 0.5 cm) and an electronic dynamometer (Yi Lian Medicine®; Shanghai, P.R. China) adjusted accordingly to provide the optimal grip span [29]. Children were asked to stand straight yet relaxed, and grip the dynamometer with the dominant hand as hard as possible for 5 sec, with the arm fully extended and without other parts of the body touching it. Each child had two tries (with a 15-sec rest in between), but only the maximum reading was recorded, to the nearest 0.1 kg. For the standing broad jump test, each child, standing behind a straight line, had two tries (with a 15-sec rest in between) to jump as far forward as possible with both legs. The longer jump was recorded to the nearest 1 cm. The distance of the jump was measured from the starting line to the heel of the most back foot.

For the measurement of body height and weight, children were asked to take off their shoes and sweater before standing on a digital weighing scale (Model RCS-150; Nantong Xineng Ltd., Jiangsu, P.R. China) or stadiometer (Nantong Xineng Ltd., Jiangsu, P.R. China) [22]. Both height and weight were recorded twice, to the nearest 0.1 cm or kg, respectively, and averaged. The body mass index (BMI) was defined as $(\text{weight in kg})/(\text{height in m})^2$; the BMI-for-age Z score (BAZ) and height-for-age Z score (HAZ) were used as indicators for wasting and stunting, respectively [30]. Thickness of skinfolds was measured at two sites, namely triceps and subscapular, with the Holtain skinfold caliper (Holtain Ltd.; Crymych, United Kingdom) [31]. Measurements were performed in triplicate to the nearest 1 mm and averaged. The sum of the mean skinfolds at both sites was used as an estimate for body fat. The hemoglobin level was measured once, to the nearest 1 g l^{-1} , with a HemoCue® Hb 301 system (HemoCue® AB.; Ängelholm, Sweden) using a drop of blood from the ear lobe. Anemia was defined according to WHO age-specific cut-offs [32].

The socioeconomic status of the participants at baseline was assessed through a questionnaire asking for the education level of the children's parents and the main source of household income. The full trial protocol is available upon request.

9.4.5. Statistical analysis

Data were entered into Excel version 2008 (Microsoft Corp.; Redmond, United States of America), double-checked, and merged into a single database for statistical analysis with STATA version 10.0 (STATA Corp.; College Station, United

States of America). The randomization code was broken after data entry and a series of internal consistency checks were completed. A per-protocol analysis was carried out in an un-blinded manner.

In the primary analysis, physical fitness and strength scores, anthropometric measurements, and hemoglobin levels were expressed as means, and changes in the means between baseline and treatment follow-ups were compared between treatment groups in a multivariate linear regression model. In a sub-analysis, changes in the means of physical fitness and strength indicators between baseline and follow-up were compared among children of distinct soil-transmitted helminth infection status regardless of treatment status. To further explore the effect of infection intensity on these measurements, distinct groups of children were identified using principal component and cluster analysis, based on species-specific soil-transmitted helminth log transformed egg counts at baseline and at the 1- and 4-month follow-ups. Changes in the means of physical fitness and strength indicators between baseline and follow-up were compared among children from six biologically meaningful groups of varying soil-transmitted helminth infection intensity.

9.5. Results

9.5.1. Baseline characteristics

As illustrated in Figure 9.1, an overall compliance of 92% was achieved with only 9 children lost to follow-ups over the 6-month trial period. Complete datasets were available for 99 children in the albendazole group and 95 children in the placebo group. No noteworthy difference in baseline socio-demographics, prevalence, and intensity of soil-transmitted helminth infections was observed between the albendazole and placebo groups (Table 9.1).

Children were also comparable in terms of physical fitness and strength at baseline. The mean VO_2 max estimate and number of 20-m laps completed were $44.9 \text{ ml kg}^{-1} \text{ min}^{-1}$ (standard deviation (SD): $2.8 \text{ ml kg}^{-1} \text{ min}^{-1}$) and 23.8 laps (SD: 8.7 laps), respectively, for the albendazole group, and $45.4 \text{ ml kg}^{-1} \text{ min}^{-1}$ (SD: $3.2 \text{ ml kg}^{-1} \text{ min}^{-1}$) and 25.2 laps (SD: 9.9 laps), respectively, for the placebo group. With regards to physical strength, the mean grip strength and standing broad jump distance were 12.6 kg (SD: 4.1 kg) and 142 cm (SD: 14 cm), respectively, for the albendazole group, and

12.6 kg (SD: 3.5 kg) and 142 cm (SD: 14 cm), respectively, for the placebo group. However, when further stratified by sex, boys had higher physical fitness and strength than girls (statistical significance achieved for all indicators except grip strength) (Table 9.1).

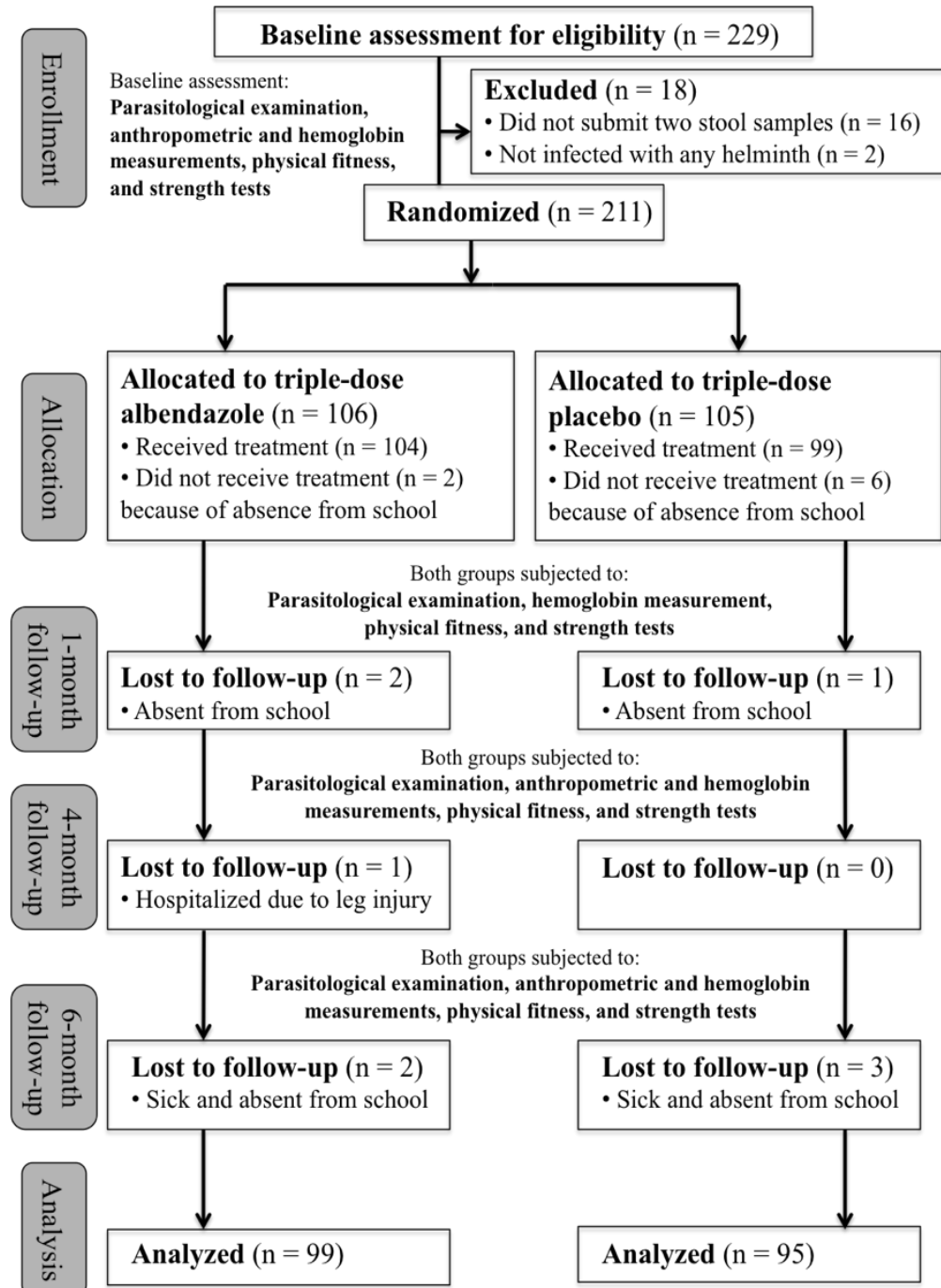


Figure 9.1. Profile of randomized controlled trial conducted in P.R. China from October 2011 to May 2012.

Table 9.1. Baseline characteristics of 194 children in south-west Yunnan province, P.R. China from October 2011 to May 2012.

Characteristics ^a	Triple-dose albendazole (n = 99)		Placebo (n = 95)	
	Male (n = 46)	Female (n = 53)	Male (n = 48)	Female (n = 47)
Age [years]	10.4 (1.1)	10.5 (1.2)	10.4 (1.0)	10.2 (1.0)
% with illiterate parents	32 (69.6%)	27 (50.9%)	28 (58.3%)	22 (46.8%)
% from family relying on farming for income	46 (100%)	53 (100%)	48 (100%)	47 (100%)
Soil-transmitted helminth prevalence				
<i>Ascaris lumbricoides</i>	46 (100%)	48 (90.6%)	43 (89.6%)	44 (93.6%)
<i>Trichuris trichiura</i>	44 (95.7%)	48 (90.6%)	47 (97.9%)	44 (93.6%)
Hookworm	31 (67.4%)	29 (54.7%)	34 (70.8%)	25 (53.2%)
<i>Taenia</i> spp.	n.r.	n.r.	1 (2.1%)	n.r.
<i>Strongyloides stercoralis</i>	n.r.	2 (3.8%)	1 (2.1%)	3 (6.4%)
Soil-transmitted helminth infection intensity				
<i>Ascaris lumbricoides</i> [EPG]	17,163 (7,548 – 59,106)	14,652 (5,922 – 51,324)	21,579 (4,653 – 43,425)	21,432 (6,390 – 65,532)
<i>Trichuris trichiura</i> [EPG]	150 (60 – 690)	222 (72 – 522)	219 (90 – 741)	318 (108 – 738)
Hookworm [EPG]	72 (0 – 204)	30 (0 – 132)	48 (0 – 126)	12 (0 – 198)
Physical fitness				
VO ₂ max estimate [ml kg ⁻¹ min ⁻¹]	45.5 (3.0)	44.4 (2.6)	45.7 (3.1)	45.0 (3.3)
20-m laps completed	25.4 (8.9)	22.4 (8.3)	27.2 (9.3)	23.2 (10.1)
Physical strength				
Grip strength [kg]	12.9 (3.7)	12.5 (4.5)	13.0 (3.5)	12.1 (3.5)
Standing broad jump distance [cm]	144 (14)	140 (14)	146 (14)	138 (13)
Anthropometric indicators				
Body height [cm]	124.8 (6.6)	129.7 (10.0)	125.6 (7.4)	127.0 (6.7)
Body weight [kg]	25.0 (3.8)	27.3 (6.1)	25.5 (4.2)	26.2 (4.7)
Body mass index [BMI; kg m ⁻²]	16.0 (1.1)	16.0 (1.4)	16.1 (1.1)	16.1 (1.6)
% wasted ^b	2 (4.4%)	2 (3.8%)	n.r.	3 (6.4%)

Article 2: Impact of deworming on physical fitness

% stunted ^c	37 (80.4%)	36 (67.9%)	39 (81.3%)	37 (78.7%)
Sum of skinfolds [mm]	10 (2)	12 (3)	10 (2)	12 (4)
Hematologic				
Hemoglobin level [g l ⁻¹]	159 (19)	162 (28)	159 (24)	155 (32)
% anemic ^d	n.r.	3 (5.7%)	1 (2.1%)	5 (10.6%)

^a Values are number of children (%) or mean (standard deviation; SD). For infection intensity, data is presented as median (interquartile range); ^b Wasting is defined as ≤ -2 BAZ score; ^c Stunting is defined as ≤ -2 HAZ score; ^d Anemia is defined according to WHO age-specific cut-offs: Hb < 115 g l⁻¹ for ages < 12 years; Hb < 120 g l⁻¹ for ages ≥ 12 and < 15 years; n.a.: not applicable; n.r.: not represented.

Treatment groups were also comparable in terms of anthropometric and hematologic characteristics at baseline. Despite the mean BMI for the albendazole and placebo group being 16.0 (SD: 1.3) and 16.1 (SD: 1.3) respectively, the baseline prevalence of wasting was only 3.6%. On the other hand, stunting was present in 76.8% (mean height for the albendazole and placebo group were 127.4 cm (SD: 8.9 cm) and 126.3 cm (SD: 7.1 cm), respectively) of the cohort. Baseline prevalence of anemia was low at 4.6%, as the mean hemoglobin levels for the albendazole and placebo group were 161 g l⁻¹ (SD: 24 g l⁻¹) and 157 g l⁻¹ (SD: 28 g l⁻¹), respectively.

9.5.2. Effect of deworming on primary and secondary outcomes

In terms of the effects of de-worming on primary outcomes, children receiving triple-dose albendazole experienced a greater change in the means of their physical fitness scores than their peers from the placebo group at all three follow-ups (Table 9.2). VO₂ max estimates increased by 1.0–2.3 ml kg⁻¹ min⁻¹ from baseline over the 6-month trial period for the albendazole group, while the increase for the placebo group ranged from 0.2–2.1 ml kg⁻¹ min⁻¹. Likewise for the number of 20-m laps completed, the range of increase was 3.4–11.9 laps and 1.4–11.7 laps for the albendazole and placebo group, respectively. When adjusted for village, and at the individual level for sex, age, height, and weight at follow-up, the difference in the increase of physical fitness between both groups was highest at the 1-month follow-up, where the increase from baseline in the albendazole group was 0.9 ml kg⁻¹ min⁻¹ ($P=0.05$) or 2.1 laps ($P=0.14$) higher than the placebo group. With regards to physical strength, the grip strength increased 0.8–2.0 kg from baseline for the albendazole group, while the placebo group experienced an increase of 0.4–1.8 kg. The difference in the increase between both groups was highest at the 1-month follow-up (0.3 kg higher in the albendazole group), but this difference was not statistically significant. The largest change in standing broad jump distance from baseline was observed among the albendazole group at the 1-month follow-up (+2 cm), but the placebo group fared better at the 6-month follow-up (+2 cm). However, both of these observations were not statistically significant.

In terms of secondary outcomes, children in the albendazole group had a larger increase, from baseline, in the means of their body height and weight and sum of skinfolds than their counterparts from the placebo group (Table 9.3). The range of

increase for body height, weight, and sum of skin folds were 2.9–3.5 cm, 1.4–2.2 kg, and 1 mm, respectively, for the albendazole group, and 2.7–3.3 cm, 1.2–1.9 kg, and 1 mm for the placebo group. However, differences between both groups in the change from baseline were statistically non-significant at all follow-ups after adjusting for sex, age at follow-up, and village. A reduction in hemoglobin level was observed in both groups at the 1- and 6-month follow-ups, and the respective reduction in the albendazole group was 2 g l⁻¹ ($P=0.72$) and 3 g l⁻¹ ($P=0.49$) higher compared to the placebo group. On the other hand, the increase from baseline in the albendazole group was 3 g l⁻¹ higher than the placebo group at the 4-month follow-up ($P=0.65$).

9.5.3. Effects of soil-transmitted helminth infections on primary outcomes

When the status of infection with soil-transmitted helminths was used as explanatory variable for the primary outcomes (Table 9.4), *T. trichiura*-infected children had 1.6 ml kg⁻¹ min⁻¹ less increase in their VO₂ max estimate from baseline than their non-infected peers at the 1-month follow-up (P -value=0.012). Similarly, hookworm-infected children had 1.1 ml kg⁻¹ min⁻¹ less increase in their VO₂ max estimate from baseline than their non-infected peers at the 6-month follow-up ($P=0.03$). In addition, the increase in the number of 20-m laps completed from baseline was 4.6 ($P=0.04$) and 6.0 ($P=0.01$) laps less for *T. trichiura*-infected children than their non-infected counterparts at the 1- and 4-month follow-ups, respectively. As further illustrated in Figure 9.2, an increase from baseline (positive change) in the number of 20-m laps completed at the 4-month follow-up was more dependent on a reduction in *T. trichiura* infection intensity than that of *A. lumbricoides* and hookworm. In terms of grip strength at the 1-month follow-up (Table 9.4), the increase from baseline among *A. lumbricoides*-infected children was 0.8 kg lower than among children not infected with this helminth species ($P=0.05$), but hookworm-infected children had 0.9 kg more increase from baseline than their non-infected peers ($P=0.04$). No statistically significant change in standing broad jump distance due to soil-transmitted helminth infection status was observed at each of the three follow-ups.

When the children were grouped according to their longitudinal infection intensity patterns, the six groups that emerged (Figure 9.3) had the following characteristics: group 1, high infection intensity of all species at all time-points; group

2, high infection intensity of all species except hookworm at all time-points; group 3, high intensity of *A. lumbricoides* re-infection by the 4-month follow-up, high infection intensity of *T. trichiura* at all time-points, and no or minimal hookworm re-infection at follow-ups; group 4, low intensity of *A. lumbricoides* re-infection by the 4-month follow-up, intermediate infection intensity of *T. trichiura* at all time-points, and no or minimal hookworm re-infection at follow-ups; group 5, intermediate intensity of *A. lumbricoides* re-infection by the 4-month follow-up, and no or minimal *T. trichiura* and hookworm re-infection at follow-ups; and group 6, infection intensity of all species increased during the follow-ups. When group 1 was used as the reference group in the multivariate linear regression models (Table 9.5), children from group 5 had consistently more increase in their VO_2 max estimate from baseline than their peers from group 1 at all follow-ups (range: 1.9-2.1 ml kg⁻¹ min⁻¹; all $P < 0.05$). A similar trend was observed for the number of 20-m laps completed and a statistically significant 5.7 more increase in the number of 20-m laps completed from baseline was noted for children from group 5 as compared to group 1 ($P=0.04$). In terms of standing broad jump distance, children from group 4 had 6 cm more increase from baseline than children from group 1 at the 4-month follow-up ($P=0.03$). No statistically significant change in grip strength dependent on soil-transmitted helminth infection intensity was observed at all follow-ups.

Table 9.2. Effects of deworming on changes in physical fitness and strength indicators (primary outcomes) at various follow-ups from baseline.

Characteristics ^a	1-month follow-up			4-month follow-up			6-month follow-up		
	ALB (n = 99)	PLB (n = 95)	Differences in Δ from baseline ^b	ALB (n = 99)	PLB (n = 95)	Differences in Δ from baseline ^b	ALB (n = 99)	PLB (n = 95)	Differences in Δ from baseline ^b
VO ₂ max estimate [ml kg ⁻¹ min ⁻¹]	45.9 (1.0)	45.5 (0.2)	0.9 (0.0 to 1.8)	45.7 (0.8)	46.2 (0.8)	0.2 (-0.8 to 1.1)	47.2 (2.3)	47.5 (2.1)	0.2 (-0.7 to 1.2)
20-m laps completed	27.2 (3.4)	26.6 (1.4)	2.1 (-0.7 to 5.0)	29.4 (5.6)	30.2 (5.0)	0.9 (-2.1 to 3.9)	35.7 (11.9)	36.9 (11.7)	0.2 (-2.7 to 3.1)
Grip strength [kg]	13.4 (0.8)	13.0 (0.4)	0.3 (-0.3 to 0.9)	14.5 (1.8)	14.2 (1.6)	0.1 (-0.5 to 0.8)	14.6 (2.0)	14.4 (1.8)	0.1 (-0.6 to 0.9)
Standing broad jump distance [cm]	142 (0)	141 (-1)	2 (-2 to 5)	146 (4)	145 (3)	1 (-2 to 4)	146 (5)	149 (7)	-2 (-5 to 2)

^a Values are mean (Δ from baseline), unless otherwise stated.

^b Differences in the changes between follow-up and baseline among the intervention groups are adjusted for village, and at the individual level for sex, age at follow-up, and height and weight at baseline (for the 1-month follow-up) or follow-up (for the 4- and 6-month follow-ups). Values are calculated from a multivariate linear regression model, presented as coefficient (95% confidence interval) and highlighted in bold if statistical significance is achieved ($P < 0.05$).

ALB: triple-dose albendazole; PLB: placebo.

Table 9.3. Effects of de-worming on changes in nutritional indicators (secondary outcomes) at various follow-ups from baseline.

Characteristics ^a	1-month follow-up			4-month follow-up			6-month follow-up		
	ALB (n = 99)	PLB (n = 95)	Differences in Δ from baseline ^b	ALB (n = 99)	PLB (n = 95)	Differences in Δ from baseline ^b	ALB (n = 99)	PLB (n = 95)	Differences in Δ from baseline ^b
Body height [cm]		n.d.		130.3 (2.9)	129.0 (2.7)	0.2 (-0.1 to 0.4)	130.9 (3.5)	129.6 (3.3)	0.2 (-0.1 to 0.4)
Body weight [kg]				27.6 (1.4)	27.0 (1.2)	0.2 (-0.1 to 0.4)	28.4 (2.2)	27.7 (1.9)	0.2 (-0.1 to 0.6)
% stunted ^c		n.d.		63 (-10.1%)	70 (-6.3%)	n.a.	66 (-7.0%)	69 (-7.4%)	n.a.
Sum of skinfolds [mm]				12 (1)	12 (1)	0 (0 to 1)	12 (1)	12 (1)	0 (0 to 1)
Hemoglobin level [g l ⁻¹]	151 (-10)	150 (-7)	-2 (-10 to 7)	164 (3)	159 (2)	3 (-8 to 13)	148 (-13)	148 (-9)	-3 (-11 to 6)

^a Values are number of children (% change from baseline) or mean (Δ from baseline), unless otherwise stated.

^b Differences in the changes between follow-up and baseline among the intervention groups are adjusted for village, and at the individual level for sex and age at follow-up. Values are calculated from a multivariate linear regression model, presented as coefficient (95% confidence interval) and highlighted in bold if statistical significance is achieved ($P < 0.05$).

^c Stunting is defined as ≤ -2 HAZ score.

^d P -value calculated from χ^2 test comparing % stunted between ALB and PLB for statistical significance.

ALB: triple-dose albendazole; PLB: placebo; n.d.: not determined.

Table 9.4. Effects of soil-transmitted helminth infection status on changes in physical fitness and strength indicators (primary outcomes) at various follow-ups from baseline.

	1-month follow-up		4-month follow-up		6-month follow-up	
Multivariate linear regression models ^a	n	Coefficient (95% CI)	n	Coefficient (95% CI)	n	Coefficient (95% CI)
(A) Change in VO₂ max estimate [ml kg⁻¹ min⁻¹]						
<i>Ascaris lumbricoides</i> -infected	97	-0.1 (-1.2 to 1.0)	167	0.7 (-0.8 to 2.2)	175	1.2 (-0.4 to 2.8)
<i>Trichuris trichiura</i> -infected	166	-1.6 (-3.0 to -0.3)	170	-1.5 (-3.1 to 0.1)	175	0.5 (-1.1 to 2.1)
Hookworm-infected	59	-0.2 (-1.5 to 1.0)	51	0.4 (-0.8 to 1.6)	56	-1.1 (-2.1 to -0.1)
(B) Change in 20-m intervals completed						
<i>Ascaris lumbricoides</i> -infected	97	-0.2 (-3.7 to 3.4)	167	3.1 (-1.4 to 7.5)	175	3.4 (-1.7 to 8.5)
<i>Trichuris trichiura</i> -infected	166	-4.6 (-8.9 to -0.3)	170	-6.0 (-10.7 to -1.2)	175	1.2 (-3.9 to 6.3)
Hookworm-infected	59	-0.1 (-4.1 to 3.9)	51	2.0 (-1.5 to 5.5)	56	-2.2 (-5.5 to 1.1)
(C) Change in grip strength [kg]						
<i>Ascaris lumbricoides</i> -infected	97	-0.8 (-1.5 to 0.0)	167	-0.5 (-1.5 to 0.5)	175	-1.2 (-2.4 to 0.1)
<i>Trichuris trichiura</i> -infected	166	0.0 (-0.9 to 0.8)	170	0.9 (-0.1 to 2.0)	175	0.7 (-0.6 to 2.0)
Hookworm-infected	59	0.9 (0.1 to 1.7)	51	0.1 (-0.7 to 0.9)	56	0.7 (-0.1 to 1.5)
(D) Change in standing broad jump distance [cm]						
<i>Ascaris lumbricoides</i> -infected	97	-2 (-6 to 2)	167	-2 (-7 to 3)	175	1 (-4 to 7)
<i>Trichuris trichiura</i> -infected	166	5 (0 to 10)	170	5 (0 to 10)	175	5 (-1 to 11)
Hookworm-infected	59	0 (-4 to 5)	51	-3 (-7 to 1)	56	1 (-3 to 5)

^a For each model, the outcome variable is highlighted with a grey bar and the explanatory variables (reference group is always not infected with the particular soil-transmitted helminth species) are presented below. All models have been adjusted for village, and at the individual level for sex, age at follow-up, and height and weight at baseline (for the 1-month follow-up) or follow-up (for the 4- and 6-month follow-ups). Values are presented as coefficient (95% confidence interval) and highlighted in bold if statistical significance is achieved ($P < 0.05$).

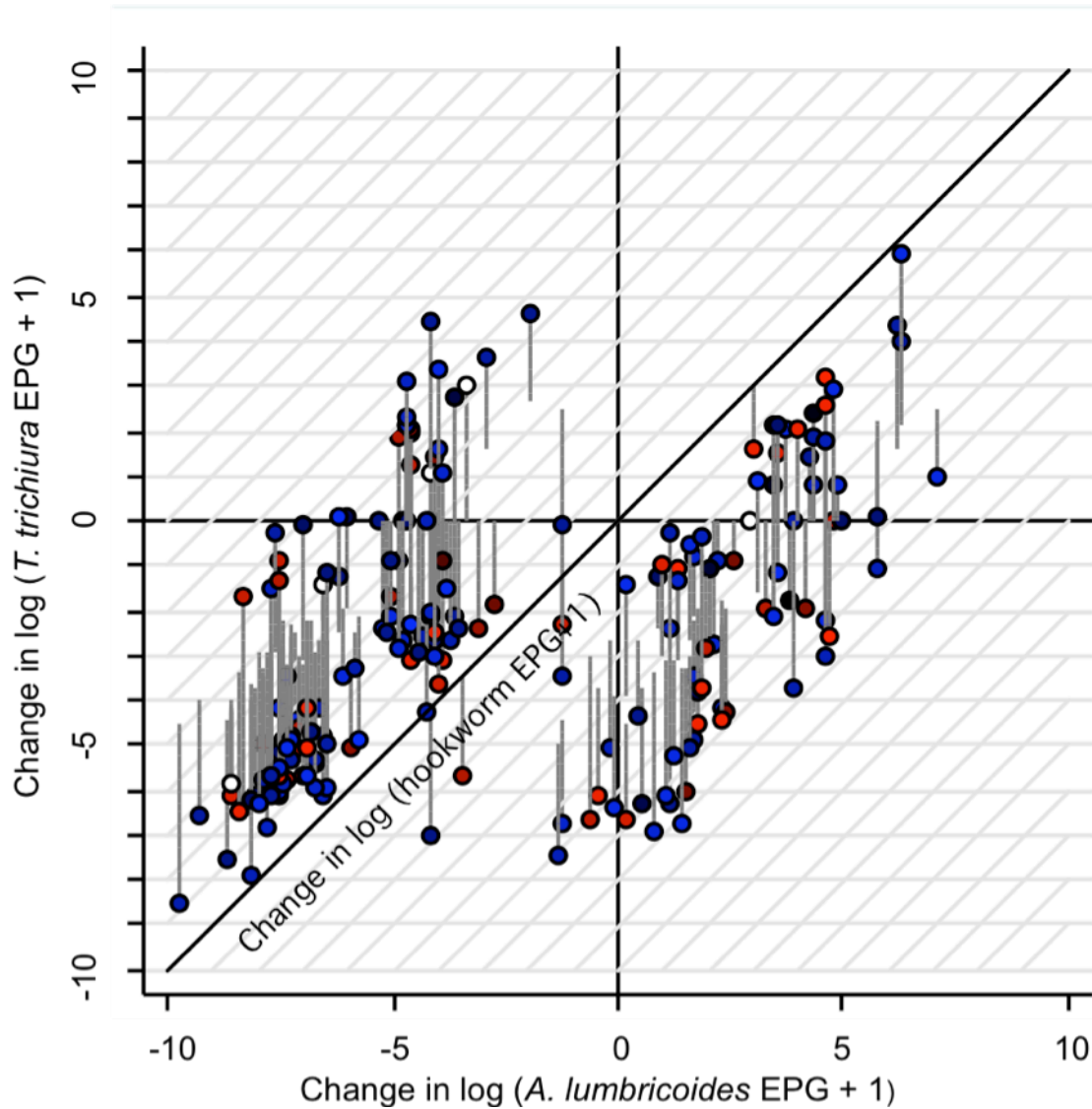


Figure 9.2. Three-dimensional visualization of changes in 20-m laps due to differences in soil-transmitted helminth infection intensities. Shown here are changes between 4-month follow-up and baseline. Blue circles indicate positive change, red circles indicate negative change and white circles indicate no change in number of 20-m intervals completed (a darker shade of colour indicates a greater degree of change).

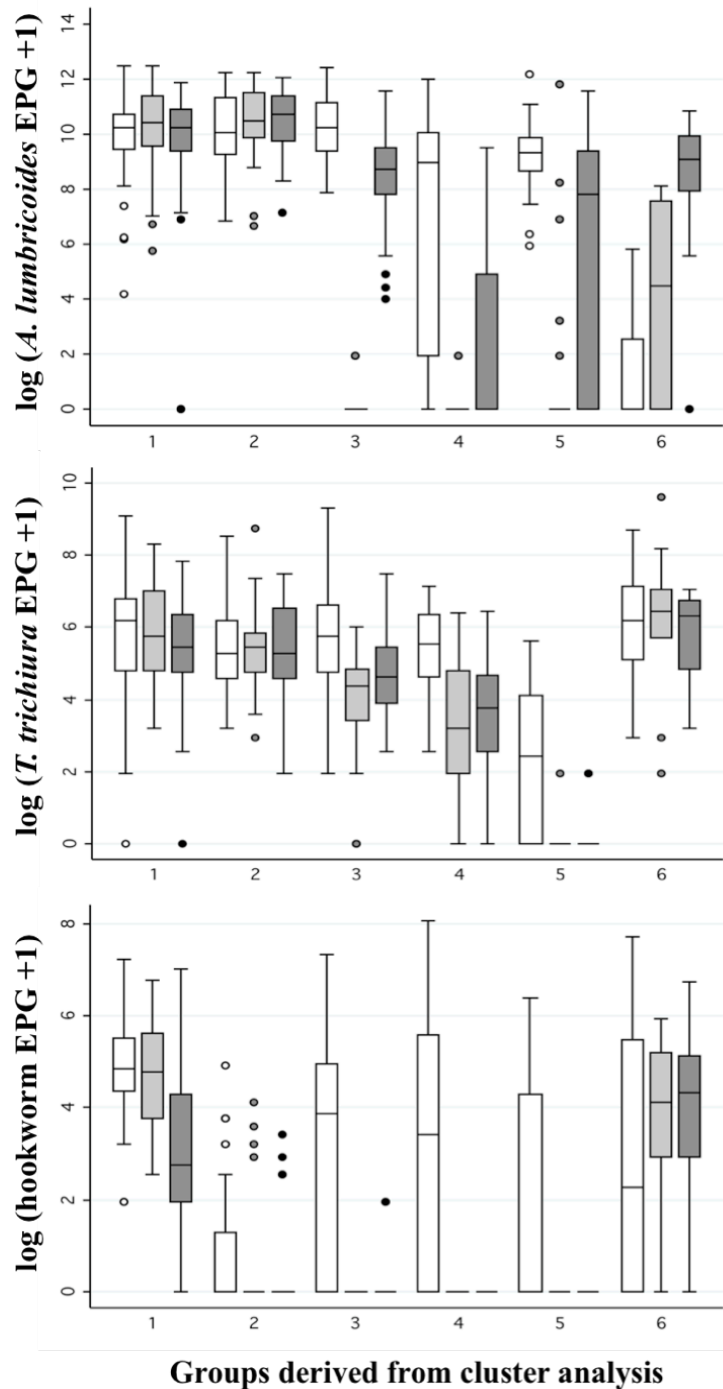


Figure 9.3. Boxplots of six infection intensity groups identified by principal component and cluster analysis. The groups are based on varying infection intensities of the three soil-transmitted helminths at baseline (white), 1-month follow-up (light grey), and 4-month follow-up (dark grey), among 194 children from a randomized controlled trial conducted in south-west Yunnan province, P.R. China.

Table 9.5. Effects of soil-transmitted helminth infection intensity on changes in physical fitness and strength indicators (primary outcomes) at various follow-ups from baseline.

	1-month follow-up	4-month follow-up	6-month follow-up
Multivariate linear regression models ^a	Coefficient (95% CI)	Coefficient (95% CI)	Coefficient (95% CI)
(A) Change in VO₂ max estimate [ml kg⁻¹ min⁻¹]			
Group 2 (n = 36)	-0.2 (-1.6 to 1.2)	-0.2 (-1.7 to 1.3)	0.7 (-0.7 to 2.1)
Group 3 (n = 53)	0.3 (-0.9 to 1.5)	-0.1 (-1.4 to 1.3)	0.1 (-1.2 to 1.4)
Group 4 (n = 27)	1.2 (-0.3 to 2.7)	0.2 (-1.5 to 1.9)	0.7 (-0.8 to 2.2)
Group 5 (n = 22)	1.9 (0.3 to 3.5)	2.1 (0.3 to 3.9)	1.9 (0.2 to 3.6)
Group 6 (n = 10)	0.3 (-1.9 to 2.4)	0.9 (-1.5 to 3.2)	0.3 (-1.9 to 2.5)
(B) Change in 20-m intervals completed			
Group 2 (n = 36)	-1.4 (-5.8 to 3.0)	-2.2 (-6.7 to 2.4)	0.5 (-4.0 to 5.0)
Group 3 (n = 53)	-0.2 (-4.1 to 3.8)	-0.8 (-4.8 to 3.3)	-1.6 (-5.6 to 2.5)
Group 4 (n = 27)	2.5 (-2.3 to 7.3)	0.3 (-4.7 to 5.3)	2.7 (-2.3 to 7.7)
Group 5 (n = 22)	5.1 (0.0 to 10.3)	5.7 (0.4 to 11.1)	3.9 (-1.5 to 9.2)
Group 6 (n = 10)	-1.7 (-8.5 to 5.1)	1.4 (-5.6 to 8.5)	0.3 (-6.7 to 7.3)
(C) Change in grip strength [kg]			
Group 2 (n = 36)	-0.6 (-1.5 to 0.3)	-0.7 (-1.7 to 0.3)	-1.2 (-2.4 to -0.1)
Group 3 (n = 53)	-0.2 (-1.1 to 0.6)	-0.1 (-1.0 to 0.8)	-0.5 (-1.5 to 0.5)
Group 4 (n = 27)	-0.4 (-1.4 to 0.7)	0.3 (-0.8 to 1.4)	-0.1 (-1.4 to 1.1)
Group 5 (n = 22)	-0.5 (-1.6 to 0.6)	-0.8 (-1.9 to 0.4)	-0.8 (-2.2 to 0.5)
Group 6 (n = 10)	0.0 (-1.5 to 1.4)	0.0 (-1.6 to 1.5)	-0.1 (-2.3 to 1.2)
(D) Change in standing broad jump distance [cm]			
Group 2 (n = 36)	-1 (-6 to 4)	1 (-4 to 6)	-2 (-7 to 3)
Group 3 (n = 53)	1 (-4 to 5)	3 (-1 to 7)	-1 (-5 to 4)
Group 4 (n = 27)	4 (-2 to 9)	6 (1 to 12)	0 (-5 to 6)
Group 5 (n = 22)	-3 (-9 to 3)	-1 (-7 to 4)	-5 (-11 to 1)
Group 6 (n = 10)	-3 (-11 to 5)	1 (-7 to 9)	1 (-7 to 9)

^a For each model, the outcome variable is highlighted with a grey bar and the explanatory variables (reference group is always group 1) are presented below. All models have been adjusted for village, and at the individual level for sex, age at follow-up, and height and weight at baseline (for the 1-month follow-up) or follow-up (for the 4-month follow-up). Values are presented as coefficient (95% confidence interval) and highlighted in bold if statistical significance is achieved ($P < 0.05$).

9.6. Discussion

As shown in our preceding work in Bulang communities [24,26], the prevalence and intensity of soil-transmitted helminth infections in this ethnic minority group can be very high. For example, in the current randomized controlled trial, we found baseline prevalence of *T. trichiura*, *A. lumbricoides*, and hookworm at 94.5%, 93.3%, and 61.3%, respectively. Therefore, an intensive de-worming regimen, consisting of triple-dose albendazole [24,33], was employed to allow children a fair chance of developing their physical fitness unaffected by intestinal helminth infections. Unexpectedly, re-infection with *A. lumbricoides* occurred rapidly and the prevalence of *A. lumbricoides* reached 80% of the pre-treatment prevalence 4 months after treatment [24]. Despite triple-dose albendazole treatment, a low cure rate of 19.6% was obtained against *T. trichiura*, corroborating previous conclusions that *T. trichiura* infection is particularly hard to cure with current anthelmintic drugs [16,34-36]. Such re-infection dynamics have complicated the evaluation of the potential health benefits of deworming and rendered the grouping of the children according to intervention near-irrelevant as the treated children might not have benefited from a meaningful helminth-free period for substantial catch-up growth. This finding further suggests that in our study area, the current WHO recommendation of single-dose albendazole (400 mg) twice yearly [15] might be insufficient in controlling soil-transmitted helminthiasis.

In a recent trial from India [37], where 1 million preschool-aged children, 1- to 6-year-old at baseline, were treated with albendazole every 6 months for 5 years, no statistically significant difference in anthropometric measurements was detected in this lightly infected population between the albendazole and control groups. In our study, even though a trend of higher values was observed among the treated cohort, no statistically significant difference in most primary and secondary outcomes between the albendazole and placebo groups was detected during the 6-month follow-up period. However, we did find one statistically significant, and biologically important difference in the VO_2 max estimate at 1-month follow-up between the albendazole and placebo groups despite the relatively small sample size.

In the sub-group analysis, we found that soil-transmitted helminth-infected children had performed significantly worse in the battery of physical fitness and strength tests than their non-infected peers. When we grouped children according to

their infection status at each follow-up, we observed that *T. trichiura*-infected children performed worse in the 20-m shuttle run than their non-infected peers. This confirmed the results from a cross-sectional survey conducted by our group where *T. trichiura*-infected children were found to complete, on average, 6.1 20-m laps less and have a VO_2 max estimate which was $1.9 \text{ ml kg}^{-1} \text{ min}^{-1}$ lower than their non-infected counterparts [22]. To survive in a host, adult *T. trichiura* worms anchor their whip-like anterior end into the wall of the large intestine and caecum by secreting pore-forming proteins. Such an invasive mechanism causes inflammation and bleeding, resulting in abdominal pain in the short term, and anemia and rectal prolapse in the long term, especially when large numbers of worms are present [38]. A significant change in physical fitness already at the 1-month follow-up could indicate that removing abdominal pain alone through the expulsion of *T. trichiura* might enhance the host's endurance in exhaustive exercises, such as the 20-m shuttle run. Hookworm-infected children were also found to have a significantly lower increase from baseline in their VO_2 max estimates than children non-infected with hookworm at the 6-month follow-up. Although anemia is a known symptom of hookworm infection and would be a plausible cause for reduced VO_2 max estimates [21], it was detected in only 10.7% of the hookworm-infected children and no significant association was found between any soil-transmitted helminth infection and hemoglobin level. The migration of the hookworm larvae through the pulmonary blood vessels, where they bore into the alveoli, could offer an alternative explanation to this observation. Although the larvae of *A. lumbricoides* undergo a similar migratory process, no reduction in VO_2 max estimates was observed in children infected with *A. lumbricoides* [38]. In terms of grip strength, the increase from baseline among *A. lumbricoides*-infected children was significantly lower, while hookworm-infected children had a higher increase from baseline, when compared to their non-infected peers. As there is currently limited evidence on the association of soil-transmitted helminth infection and grip strength, these inconsistent findings warrant further investigation.

When children were grouped according to infection intensity, we were able to take into consideration the degree of infection at baseline, 1- and 4-month follow-ups, and the extent of multiparasitism for each child. These analyses revealed that individuals with a combination of no or minimal *T. trichiura* and hookworm re-

infection achieved higher improvements during the follow-ups in the 20-m shuttle run, as compared to peers with high infection intensity of all species. In addition, children with no or minimal *A. lumbricoides* and hookworm re-infection performed better in the standing broad jump than their counterparts with high infection intensity of all species. These findings provide further evidence of the impact of soil-transmitted helminth infections on the physical fitness and strength of school-aged children.

The anthropometric and physical strength findings from this trial should be viewed in the light of the following limitations. A follow-up period of 6 months is too short for an accurate evaluation of anthropometric gains and physical strength increments from longer-term physical growth due to deworming. Taking into account that keeping controls untreated for a long period would be difficult based on ethical considerations, a 3- to 5-year prospective cohort study, where children are treated regularly to ensure that they are helminth-free, and the changes in anthropometric indicators and physical strength from baseline are monitored and compared with changes in soil-transmitted helminth infection intensity over time, could be a more appropriate study design. Finally, catch-up growth after anthelmintic treatment can only occur if the diet is sufficient [39]. Based on the investigators' observations in the field, most of the children's diet consists mainly of white rice with little protein sources. Dietary improvements, in addition to deworming, are therefore necessary in the current setting and should be considered in future studies.

We conclude that there is no strong evidence for significant improvements in physical fitness and anthropometric indicators due to deworming with triple-dose albendazole. This might be partly explained by the rapid re-infection observed with *A. lumbricoides* and low cure rates with *T. trichiura*. However, negative impacts on the physical fitness and strength were observed in school-aged children infected with soil-transmitted helminths in sub-group analyses. In particular, the clear effects of *T. trichiura* infection on physical fitness in this trial is intriguing as the public health burden of this helminth species is currently not as well defined as the other two species. The fact that *T. trichiura* infection had the strongest negative impact on the physical fitness of the children, but was hardly cured with triple-dose albendazole is another major concern. Finally, we also showed that the morbidities observed were infection intensity-dependent and in order to control them, regular deworming,

coupled with dietary improvements, improvements in water, sanitation, and hygiene, should be considered.

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9.8. References

1. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, et al. (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 367: 1521-32.
2. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Ehrlich Sachs S, et al. (2007) Control of neglected tropical diseases. *N Engl J Med* 357: 1018-27.
3. Pullan RL, Brooker SJ. (2012) The global limits and population at risk of soil-transmitted helminth infections in 2010. *Parasit Vectors* 5: 81.
4. Barry MA, Simon GG, Mistry N, Hotez PJ. (2013) Global trends in neglected tropical disease control and elimination: impact on child health. *Arch Dis Child* 98: 635-41.
5. Brooker S, Clements ACA, Bundy DAP. (2006) Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol* 62: 221-61.
6. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. (2008) Helminth infections: the great neglected tropical diseases. *J Clin Invest* 118: 1311-21.
7. Stephenson LS, Latham MC, Ottesen EA. (2000) Malnutrition and parasitic helminth infections. *Parasitology* 121 Suppl: S23-38.
8. Crompton DWT, Nesheim MC. (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr* 22: 35-59.
9. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, et al. (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380: 2197-223.
10. Niederer I, Kriemler S, Gut J, Hartmann T, Schindler C, et al. (2011) Relationship of aerobic fitness and motor skills with memory and attention in preschoolers (Ballabeina): a cross-sectional and longitudinal study. *BMC Pediatr* 11: 34.
11. Bass RW, Brown DD, Laurson KR, Coleman MM. (2013) Physical fitness and academic performance in middle-school students. *Acta Paediatr* 102: 832-7.
12. Fenwick A, Figenschou BH. (1972) The effect of *Schistosoma mansoni* infection of the productivity of cane cutters on a sugar estate in Tanzania. *Bull World Health Organ* 47: 567-72.
13. Hotez P. (2008) Hookworm and poverty. *Ann N Y Acad Sci* 1136: 38-44.

14. Smith JP. (1999) Healthy bodies and thick wallets: the dual relation between health and economic status. *J Econ Perspect* 13: 144-66.
15. WHO. Preventive chemotherapy in human helminthiasis: coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme managers. Geneva: World Health Organization; 2006.
16. Keiser J, Utzinger J. (2008) Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* 299: 1937-48.
17. Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P. (2012) Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin and school performance. *Cochrane Database Syst Rev* 11: CD000371.
18. Nagpal S, Sinclair D, Garner P. (2013) Has the NTD community neglected evidence-based policy? *PLoS Negl Trop Dis* 7: e2238.
19. Stephenson LS, Latham MC, Kinoti SN, Kurz KM, Brigham H. (1990) Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg* 84: 277-82.
20. Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. (1993) Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr* 123: 1036-46.
21. Bustinduy AL, Thomas CL, Fiutem JJ, Parraga IM, Mungai PL, et al. (2011) Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric. *PLoS Negl Trop Dis* 5: e1213.
22. Yap P, Du ZW, Chen R, Zhang LP, Wu FW, et al. (2012) Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. *Parasit Vectors* 5: 50.
23. Müller I, Coulibaly JT, Fürst T, Knopp S, Hattendorf J, et al. (2011) Effect of schistosomiasis and soil-transmitted helminth infections on physical fitness of school children in Côte d'Ivoire. *PLoS Negl Trop Dis* 5: e1239.
24. Yap P, Du ZW, Wu FW, Jiang JY, Chen R, et al. (2013) Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in Yunnan, People's Republic of China. *Am J Trop Med Hyg* 89: 23-31.
25. Steinmann P, Zhou XN, Du ZW, Jiang JY, Xiao SH, et al. (2008) Tribendimidine and albendazole for treating soil-transmitted helminths, *Strongyloides stercoralis* and *Taenia* spp.: open-label randomized trial. *PLoS Negl Trop Dis* 2: e322.

26. Steinmann P, Du ZW, Wang LB, Wang XZ, Jiang JY, et al. (2008) Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. *Am J Trop Med Hyg* 78: 760-9.
27. Yap P, Fürst T, Müller I, Kriemler S, Utzinger J, Steinmann P. (2012) Determining soil-transmitted helminth infection status and physical fitness of school-aged children. *J Vis Exp*: e3966.
28. Léger LA, Mercier D, Gadoury C, Lambert J. (1988) The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci* 6: 93-101.
29. España-Romero V, Artero EG, Santaliestra-Pasias AM, Gutierrez A, Castillo MJ, Ruiz JR. (2008) Hand span influences optimal grip span in boys and girls aged 6 to 12 years. *J Hand Surg Am* 33: 378-84.
30. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. (2007) Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* 85: 660-7.
31. Kriemler S, Puder J, Zahner L, Roth R, Meyer U, Bedogni G. (2010) Estimation of percentage body fat in 6- to 13-year-old children by skinfold thickness, body mass index and waist circumference. *Br J Nutr* 104: 1565-72.
32. WHO. Assessing the iron status of populations: report of a joint World Health Organization/Centers for Disease Control and Prevention: technical consultation on the assessment of iron status at the population level. Geneva: World Health Organization; 2007.
33. Steinmann P, Utzinger J, Du ZW, Jiang JY, Chen JX, et al. (2011) Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp.: a randomized controlled trial. *PLoS One* 6: e25003.
34. Knopp S, Mohammed KA, Speich B, Hattendorf J, Khamis IS, et al. (2010) Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial. *Clin Infect Dis* 51: 1420-8.
35. Keiser J, Utzinger J. (2010) The drugs we have and the drugs we need against major helminth infections. *Adv Parasitol* 73: 197-230.
36. Speich B, Ame SM, Ali SM, Alles R, Hattendorf J, et al. (2012) Efficacy and safety of nitazoxanide, albendazole, and nitazoxanide-albendazole against *Trichuris trichiura* infection: a randomized controlled trial. *PLoS Negl Trop Dis* 6: e1685.
37. Awasthi S, Peto R, Read S, Richards SM, Pande V, Bundy D. (2013) Population deworming every 6 months with albendazole in 1 million pre-school children in north India: DEVTA, a cluster-randomised trial. *Lancet* 381: 1478-86.

38. Hall A, Hewitt G, Tuffrey V, de Silva N. (2008) A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Matern Child Nutr* 4 Suppl 1: 118-236.
39. Hall A. (2007) Micronutrient supplements for children after deworming. *Lancet Infect Dis* 7: 297-302.

10. Visualizing the impact of soil-transmitted helminth infections on the physical fitness levels of children in the People's Republic of China

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10.1. Abstract

Soil-transmitted helminth infections remain an important public health problem among many rural populations in the People's Republic of China (P.R. China). According to the most recent national survey in 2001-2004, the prevalence rates for *A. lumbricoides*, hookworm and *T. trichiura* were 12.7%, 6.1% and 4.6%, respectively, translating into an estimated 154 million infections, many of them among children. The aim of this study was to predict and visualize the impact of soil-transmitted helminth infections on the physical fitness of children in P.R. China. Two data sources were employed for this study, namely an extensive georeferenced soil-transmitted helminth prevalence database, and data from a randomized controlled trial that generated data on the parasitological status and physical fitness levels of children. The change in physical fitness over 1 month (p) across P.R. China was predicted for a smooth surface of soil-transmitted helminth risk (r) with the following equation: $Y_p = \beta_p * X_r + \beta_{sex} * 0.461 + \beta_{age} * 11 + \text{constant}$. The largest depression of increase of physical fitness over 1 month due to soil-transmitted helminths, in particular *T. trichiura* infections, were observed in the rural areas of Hainan, Jiangxi, Shandong, Guizhou, Sichuan and Yunnan provinces. Upon population-adjustments for the proportion of 0-14 year olds, several provinces including Hubei and the coastal areas of Fujian and Guangdong showed increased reductions in the gain of physical fitness over 1 month. This study highlights the costs of soil-transmitted helminth infections in terms of an individual's health, which eventually could translate into reduced economic output. Visualizing this burden should facilitate advocating for the implementation of a comprehensive national soil-transmitted helminth control programme.

10.2. Background

Soil-transmitted helminths, namely *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*), are currently estimated to infect around 1 billion people, while 5 billion people are at risk of infection worldwide [1]. They are endemic in impoverished populations, mainly in the Americas, Asia and sub-Saharan Africa. Despite the tremendous economic and social advances made by the People's Republic of China (P.R. China) over the past decades, soil-transmitted helminth infections remain an important public health problem among some of its rural populations. According to the national survey conducted from 2001 to 2004 [2], the prevalences of *A. lumbricoides*, hookworm and *T. trichiura* were 12.7%, 6.1% and 4.6%, respectively, translating into an estimated 154 million infections, many of them children. Although the overall prevalence of soil-transmitted helminths had decreased by 61-74% compared to the previous national survey conducted in the early 1990s, hotspots of infection still persists in peripheral provinces and autonomous regions such as Hainan, Guizhou, and Yunnan [2,3].

Globally, infected populations are burdened with approximately 5.2 million disability-adjusted life years (DALYs). Sub-clinical morbidities, ranging from anemia to a lack of cognitive and physical development, encompass most of the burden [4-6]. A cross-sectional study implemented by our group in Yunnan province, P.R. China, demonstrated that the physical fitness of children infected with *T. trichiura* was significantly lower than that of their non-infected peers [3]. A randomized controlled trial conducted subsequently to further explore this association found that compared to the baseline, the physical fitness of *T. trichiura*-infected children had increased significantly less at the 1-month follow-up than that of non-*T. trichiura*-infected children [4]. Evidence from the above-mentioned and other epidemiological studies [5-7] led us to hypothesize that a significant yet currently neglected or misattributed burden of soil-transmitted helminth infections stems from a reduction of physical fitness in infected children. It has been suggested that soil-transmitted helminth infections can cause nutrient loss and decreased food intake, leading to reduced growth in children [8,9]. This diminished growth probably causes a depression in physical fitness levels [8]. Hookworm-induced anaemia can also cause fatigue, shortness of breath and decreased energy that will translate into reduced physical fitness [7]. A reduction in physical fitness, in turn, has a direct impact on the general

health and productivity of a population, not least in agricultural/labour-based communities typical of developing societies like rural P.R. China.

The aim of this study was to predict and visualize the impact of soil-transmitted helminth infections on the physical fitness of children in P.R. China based on the results from a randomized, placebo-controlled study and an extensive database of soil-transmitted helminth prevalence surveys.

10.3. Methods

10.3.1. Data sources

The two major data sources were: (i) the soil-transmitted helminth prevalence database, which contains geographically referenced soil-transmitted helminth survey details, including the number of infected individuals among all examined, stratified by soil-transmitted helminth species, age and sex whenever possible, and the diagnostic techniques, from more than 900 distinct locations in 22 provinces, 5 autonomous regions and 4 municipalities. These data were obtained through a systematic review of the Chinese and international scientific literature (peer-reviewed journals and ‘grey literature’) and all data extracted were stored in a MySQL database with a web interface allowing for free database access and management [10]; and (ii) data on the parasitological status and physical fitness level (assessed with the 20-m shuttle run test and presented as the estimate of the maximum volume of oxygen utilized by the body in 1 min of exhaustive exercise (VO_2 max estimate; $\text{ml}^{-1}\text{kg}^{-1}\text{min}$) and the number of 20-m intervals completed) for approximately 200 children, aged 9-12 years, obtained through a series of follow-ups in a randomized controlled trial [4].

10.3.2. Statistical analysis, prediction and mapping

For each soil-transmitted helminth species, linear regressions, adjusted for age and sex, where the outcome was the change in physical fitness over 1 month and the covariate was the infection status (non-infected versus infected), was performed in STATA version 10.1 (STATA Corp., College Station, TX).

Using the coefficients obtained from the linear regression models, the change in physical fitness over 1 month (p) across P.R. China was predicted over a smooth surface of soil-transmitted helminth risk (r) with the following equation: $Y_p = \beta_p * X_r +$

$\beta_{\text{sex}}*0.461 + \beta_{\text{age}}*11 + \text{constant}$ (Y_p intercept). A total of 4 main maps, namely the depression in the increase of (i) VO_2 max estimates or (ii) the number of 20-m intervals completed over a 1-month interval due to the presence of any soil-transmitted helminths and depression in the increase of (iii) VO_2 max estimate or (iv) number of 20-m intervals completed over 1 month due to the presence of *T. trichiura* only, were created. For each of these maps, outlines of the lower and upper boundaries of the predicted depressions as well as the population-adjusted (for the proportion of 0-14 year olds in P.R. China) estimates were obtained.

10.4. Results

Linear regression models, adjusted for age and sex, revealed that when compared to their non-infected peers, children infected with soil-transmitted helminths had a $1.44 \text{ ml}^{-1}\text{kg}^{-1}\text{min}$ lower increase in their VO_2 max estimates ($P = 0.041$) and a 3.45 lower increase in the number of 20-m intervals ($P = 0.128$) completed over a 1-month period. In addition, children infected with *T. trichiura* had $1.81 \text{ ml}^{-1}\text{kg}^{-1}\text{min}$ less increase in VO_2 max estimates ($P = 0.005$) and 4.97 less increase in the number of 20-m intervals ($P = 0.015$) completed over 1 month than children not infected with *T. trichiura*.

As illustrated in Figures 10.1-10.4, the largest reductions, represented by red and orange shades, in the increase of physical fitness over 1 month due to soil-transmitted helminth, and *T. trichiura* infections in particular, were observed in the provinces, namely of Hainan, Jiangxi, Shandong, Guizhou, Sichuan and Yunnan. However, when the estimates were adjusted for the proportion of 0-14 year olds, additional provinces, e.g. Hubei, and the coastal areas of Fujian and Guangdong, showed increased reductions in the gain of physical fitness over the 1-month period. The same trends were observed for both VO_2 max estimates and the number of 20-m intervals completed. There were no significant differences between the physical fitness reductions due to soil-transmitted helminths and *T. trichiura* alone.

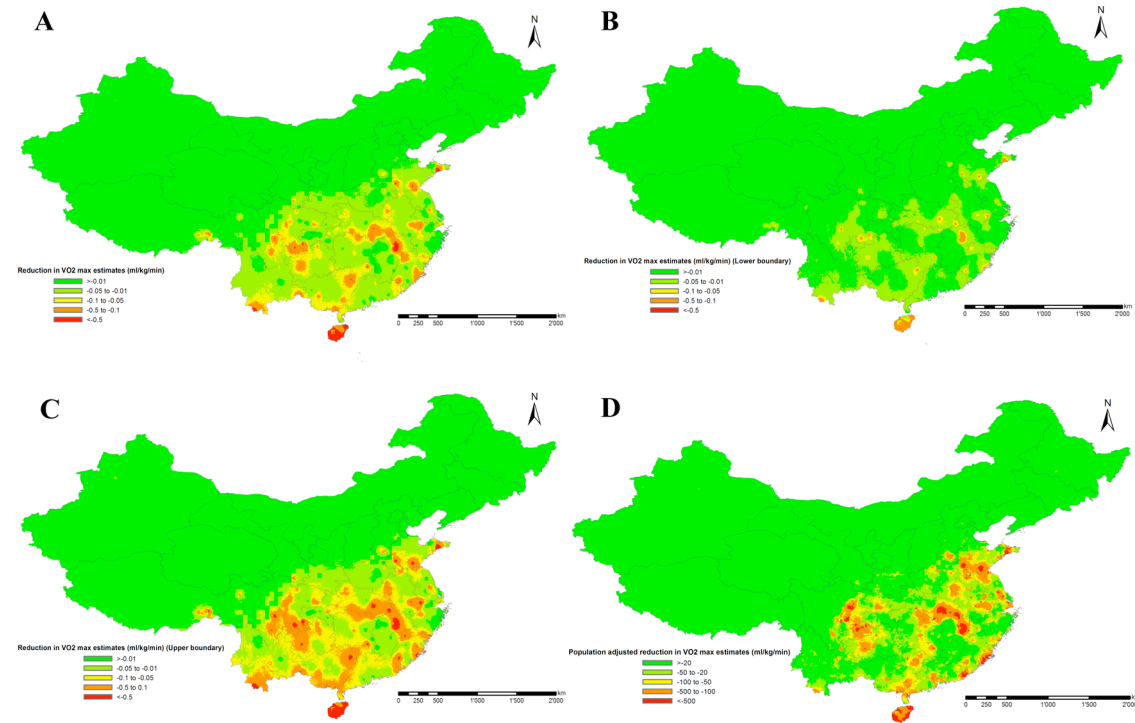


Figure 10.1. Depression of the increase of VO₂ max estimates over 1 month due to the presence of soil-transmitted helminths (A), with lower (B) and upper (C) boundaries, and population adjusted estimates (D).

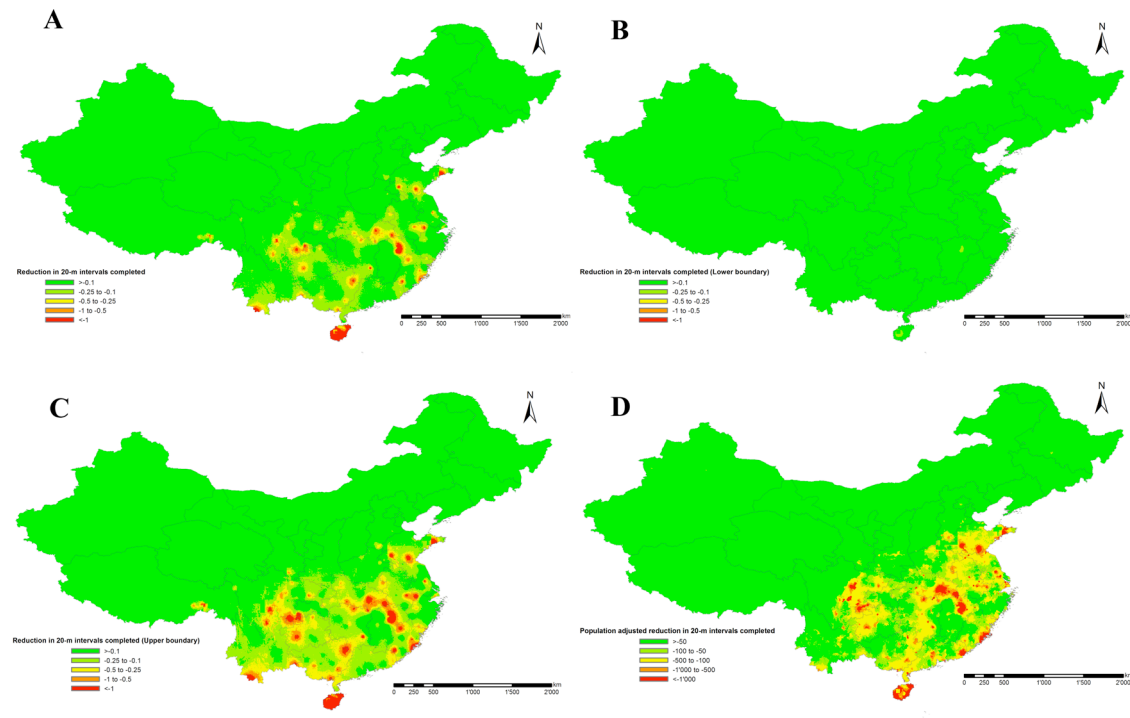


Figure 10.2. Depression of the increase of the number of 20-m intervals completed over 1 month due to the presence of soil-transmitted helminths (A), with lower (B) and upper (C) boundaries, and population adjusted estimates (D).

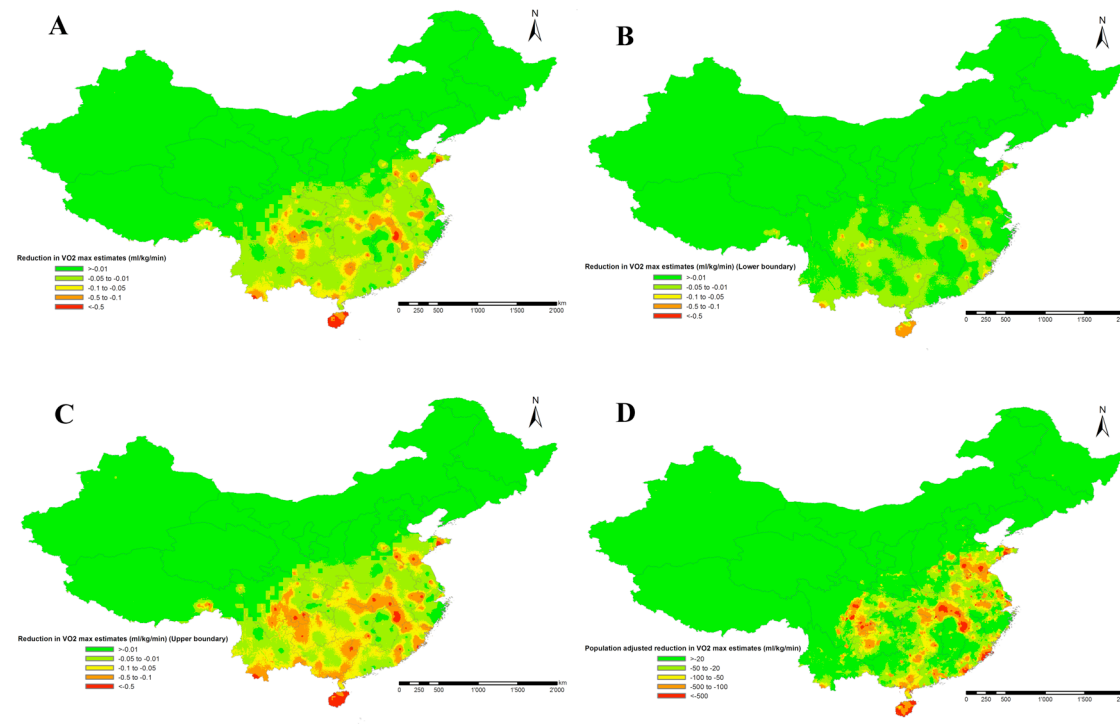


Figure 10.3. Depression of the increase of VO₂ max estimates over 1 month due to the presence of *T. trichiura* only (A), with lower (B) and upper (C) boundaries, and population adjusted estimates (D).

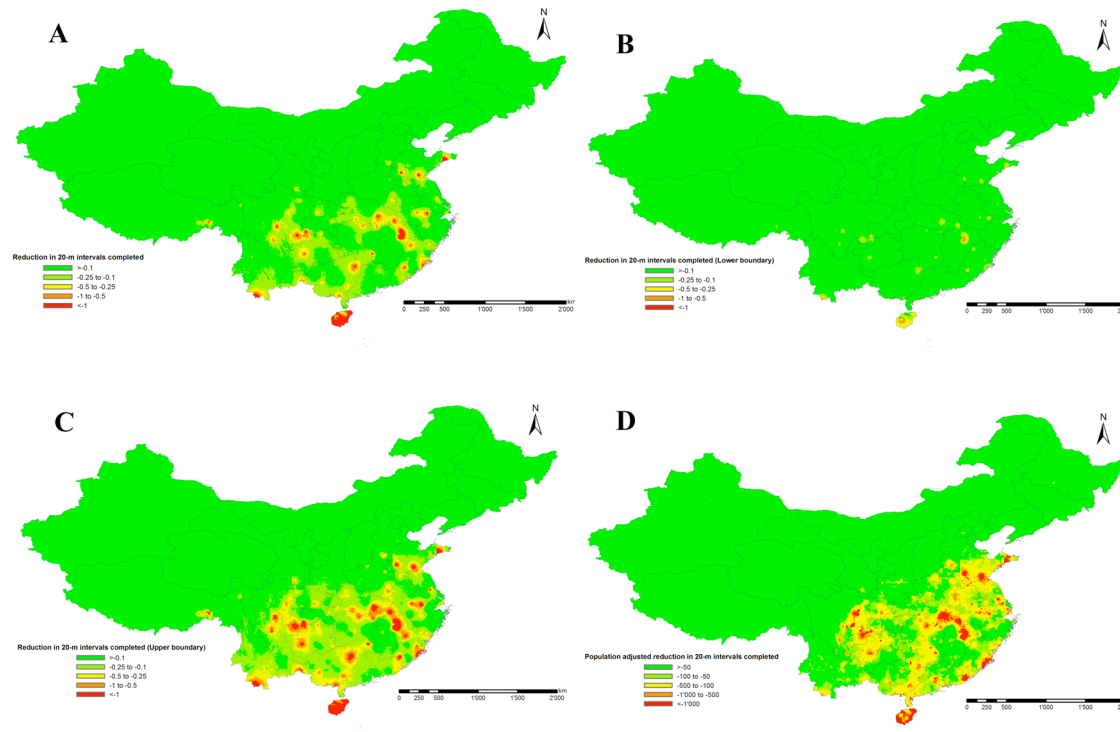


Figure 10.4. Depression of the increase of the number of 20-m intervals completed over 1 month due to the presence of *T. trichiura* only (A), with lower (B) and upper (C) boundaries, and population adjusted estimates (D).

10.5. Discussion

The impact of soil-transmitted helminth infections on the development of physical fitness levels of children over a 1month period was estimated and visualized across P.R. China. From a global scientific perspective, focusing on physical fitness puts an emphasis on a mostly overlooked, yet potentially huge, component of the emerging picture of the full spectrum of morbidities due to soil-transmitted helminth infections [11,12].

According to the soil-transmitted helminth prevalence database, the majority of these infections are located in the provinces of Hainan, Jiangxi, Guizhou, Sichuan and Yunnan [10]. Given that these infections were the major predictors of physical fitness levels in our model, the aforementioned provinces also saw the greatest impact on the gains in VO₂ max estimates and number of 20-m intervals completed over 1 month. In more densely populated provinces, this public health burden is heavier as illustrated by the population-adjusted estimates. Reduced physical fitness not only affects the physical performance of the children, it also influences their academic achievements [13]. Furthermore, the populations affected are often located in rural areas, where a majority of the jobs are labor intensive, particularly in agriculture, and thus, children growing up with reduced physical fitness might eventually become less productive adults.

By comparing figures 1-2 with figures 3-4, it becomes clear that among the three soil-transmitted helminth species, *T. trichiura* infections account for the greatest share of the burden from depressed physical fitness levels of children. Given that these infections cause inflammation and bleeding in the large intestine [14], it is indeed conceivable that such infections could lower the performance of children in physically exhaustive tests, such as the 20-m shuttle run.

In a next step, the predictions presented in this study require field validation, whereby the actual physical fitness of children is measured across P.R. China, e.g. through random sampling followed by testing of physical fitness. Collecting more empirical evidence would allow better assessment of the extent to which this particular morbidity contributes to the overall public health burden due to soil-transmitted helminth infections.

The current maps show that significant reductions in the physical fitness of children could result from soil-transmitted helminth infections, especially those due to

T. trichiura, in high-endemicity areas. Still, we acknowledge that the current model might be overly simplistic and its accuracy is limited by the omission of additional risk factors and explanatory variables, such as anthropometric indicators and haemoglobin levels, which are also known to have an effect on physical fitness. In order to address these limitations, a systematic review of studies measuring anthropometric indicators and haemoglobin level in school-aged children across P.R. China could be performed and a geographically referenced nutritional database, akin to the soil-transmitted helminth database, established. Using this national nutritional database, we could then further develop the geo-statistical model to predict the impact of soil-transmitted helminth infections and nutritional indicators on the physical fitness of children, resulting in predictions of higher accuracy.

In conclusion, this study highlights the potential cost that soil-transmitted helminth infections have in terms of an individual child's health, which could eventually translate into lost economic opportunities. This further stresses the need to finally implement the long-overdue comprehensive national soil-transmitted helminth control programme [15,16]. Furthermore, the routine and standardized measurement and publication of variables, such as infection intensity and anthropometric indicators, should be promoted, so that more accurate Bayesian-based prediction and mapping could be undertaken.

10.6. Acknowledgments

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10.7. References

1. Pullan RL, Brooker SJ. The global limits and population at risk of soil-transmitted helminth infections in 2010. *Parasit Vectors* 2012; 5:81.
2. Coordinating Office of the National Survey on the Important Human Parasitic Diseases. [A national survey on current status of the important parasitic diseases in human population]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 2005; 23(5 Suppl):332-40.
3. Yap P, Du ZW, Chen R, Zhang LP, Wu FW, Wang J, et al. Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. *Parasit Vectors* 2012; 5:50.
4. Yap P, Wu FW, Du ZW, Chen R, Jiang JY, Wang J, et al. Effect of de-worming on the physical fitness of children in Yunnan, China: a double blind, randomized controlled trial. *PLoS Negl Trop Dis* 2013; Submitted.
5. Stephenson LS, Latham MC, Kinoti SN, Kurz KM, Brigham H. Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg* 1990; 84(2):277-82.
6. Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr* 1993; 123(6):1036-46.
7. Bustinduy AL, Thomas CL, Fiutem JJ, Parraga IM, Mungai PL, Muchiri EM, et al. Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric. *PLoS Negl Trop Dis* 2011; 5(7):e1213.
8. Stephenson LS, Latham MC, Ottesen EA. Malnutrition and parasitic helminth infections. *Parasitology* 2000; 121 Suppl:S23-38.
9. Crompton DWT, Nesheim MC. Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr* 2002; 22:35-59.
10. Lai YS, Zhou XN, Utzinger J, Vounatsou P. Bayesian geostatistical modelling of soil-transmitted helminth survey data in the People's Republic of China. *Parasit Vectors* 2013; In press.
11. Chan MS. The global burden of intestinal nematode infections--fifty years on. *Parasitol Today* 1997; 13(11):438-43.
12. WHO. The global burden of disease: 2004 update. Geneva, Switzerland: World Health Organization; 2008.

13. Castelli DM, Hillman CH, Buck SM, Erwin HE. Physical fitness and academic achievement in third- and fifth-grade students. *J Sport Exerc Psychol* 2007; 29(2):239-52.
14. Hall A, Hewitt G, Tuffrey V, de Silva N. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Matern Child Nutr* 2008; 4 Suppl 1:118-236.
15. Montresor A, Cong DT, Sinuon M, Tsuyuoka R, Chanthavisouk C, Strandgaard H, et al. Large-scale preventive chemotherapy for the control of helminth infection in Western Pacific countries: six years later. *PLoS Negl Trop Dis* 2008; 2(8):e278.
16. Li T, He S, Zhao H, Zhao G, Zhu XQ. Major trends in human parasitic diseases in China. *Trends Parasitol* 2010; 26(5):264-70.

11. Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in Yunnan, People's Republic of China

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11.1. Abstract

Post-treatment soil-transmitted helminth re-infection patterns were studied as part of a randomized controlled trial among school-aged children from an ethnic minority group in Yunnan province, People's Republic of China. Children with a soil-transmitted helminth infection (n = 194) were randomly assigned to triple-dose albendazole or placebo and their infection status monitored over a 6-month period using the Kato-Katz and Baermann techniques. Baseline prevalences of *Trichuris trichiura*, *Ascaris lumbricoides*, hookworm, and *Strongyloides stercoralis* were 94.5%, 93.3%, 61.3%, and 3.1% respectively, with more than half harboring triple-species infections. For the intervention group (n = 99), the 1-month post-treatment cure rates were 96.7%, 91.5%, and 19.6% for hookworm, *A. lumbricoides*, and *T. trichiura*, respectively. Egg reduction rates were above 88% for all three species. Rapid re-infection with *A. lumbricoides* was observed: the prevalence 4 and 6 months post-treatment was 75.8% and 83.8%, respectively. Re-infection with hookworm and *T. trichiura* was considerably slower.

11.2. Introduction

More than 1 billion people are infected with soil-transmitted helminths, i.e., *Ascaris lumbricoides*, *Trichuris trichiura*, and the two hookworm species, *Ancylostoma duodenale* and *Necator americanus*.¹ The collective global burden due to soil-transmitted helminthiasis is estimated at 5.2 million disability-adjusted life years (DALYs).² Yet, soil-transmitted helminthiasis belongs to the group of infectious diseases commonly referred to as neglected tropical diseases.^{3,4} While soil-transmitted helminth infections have largely disappeared from developed countries following sustained improvements in living and hygiene conditions,⁵ they continue to thrive where broad socio-economic development is elusive. Specifically, they are still common among the world's poorest populations in the Americas, Asia, and sub-Saharan Africa that lack access to clean water and improved sanitation, and where hygiene is poor.^{6,7} Children and pregnant women are most vulnerable to the detrimental effects of soil-transmitted helminth infections, among them anemia, nutritional deficiencies, and impairments in cognitive and physical development.⁸⁻¹¹

Preventive chemotherapy, i.e., the periodic administration of anthelmintic drugs to entire populations or high-risk groups (e.g., school-aged children), is the hallmark of the control strategy currently advocated by the World Health Organization (WHO). Single-dose albendazole (400 mg) or mebendazole (500 mg) are used in virtually all preventive chemotherapy schemes against soil-transmitted helminthiasis.¹²⁻¹⁴ While single-dose albendazole is highly efficacious against *A. lumbricoides* and reasonably so against hookworm, single-dose mebendazole reliably cures only *A. lumbricoides* infections but is considerably less efficacious against hookworm. The treatment of *T. trichiura* with a single dose of either drug results in low cure rates.¹⁵ As both drugs are rapidly cleared from the human body and thus do not confer protection against re-infection, other control strategies, such as health education, provision of clean water and improved sanitation, and shoe wearing are required to lower infection levels permanently. Indeed, a recent systematic review and meta-analysis has shown that post-treatment re-infection is common, with prevalences of *A. lumbricoides* and *T. trichiura* returning to almost pre-treatment levels within 12 months of drug administration.¹⁶ Proponents of preventive chemotherapy argue that this is acceptable as the primary aim of this strategy is to reduce infection intensity and thus, lower or eliminate morbidity from chronic helminth infections.¹⁷ Indeed, infection intensities usually recover much slower than prevalences

following treatment. However, the long-term health impact and sustainability of preventive chemotherapy still need further investigation.¹⁸ The potential development and spread of drug resistance is another concern.¹⁹

We conducted a randomized controlled trial in Yunnan province, People's Republic of China (P.R. China), investigating the effects of triple-dose albendazole on physical fitness of school-aged children. We observed unexpectedly rapid re-infection with soil-transmitted helminths, and hence decided to report these findings (secondary outcomes of the trial) ahead of the primary outcomes, due to their public health importance.

11.3. Materials and methods

11.3.1. Study location and participants

In a first step, a rapid appraisal was carried out to identify village schools where the prevalence of any soil-transmitted helminth was 70% or higher. The study finally included participants from five village schools located in the mountainous Bulangshan township, a sub-division of Menghai county in Xishuangbanna Dai Autonomous prefecture, situated in Yunnan province, P.R. China. The field work was carried out between October 2011 and May 2012. The five village schools are from: (i) Sandui (geographical coordinates: 21°33'07'' N latitude and 100°19'34'' E longitude; altitude: 1,566 m above sea level (asl)); (ii) Kongkan (21°32'34'' N and 100°20'25'' E; 1,195 m asl); (iii) Laozhai (21°31'37'' N and 100°18'01'' E; 1,399 m asl); (iv) Laonandong (21°33'28'' N and 100°21'45'' E; 1,188 m asl); and (v) Mannuo (21°33'27'' N and 100°23'53'' E; 1,352 m asl).

The county borders Myanmar and the study area is inhabited by the Bulang ethnic minority group. The sub-tropical climate is characterized by arid and cool winters, while summers are hot and rainy. Adult literacy rates are relatively low. Today, most children attend at least primary school, albeit often only for some years. Boys commonly receive part of their education in temple schools. Modern health care is lacking at village level, as only grassroots “doctors” with none or minimal formal medical training are present. Government-led health care facilities are located about 20 km away in the Bulangshan township. Water supply in the villages is piped, but water sources are unprotected and the water untreated. Power outages are common. Sanitation infrastructure is generally

unavailable and open defecation takes place around the house or at the fringe of the village. Prior to our study, no survey or control activities targeting soil-transmitted helminthiasis have been implemented in the study villages. The epidemiology and control of soil-transmitted helminthiasis in other broadly similar Bulang communities have been studied by our group, with key findings reported elsewhere.^{11,20-22}

Children aged 9–12 years were recruited from the village primary schools for a baseline screening for soil-transmitted helminth infections. All children attending school and falling into this age bracket, who had written informed consent from their parents/guardians, were invited to participate. The following inclusion criteria were applied in the randomized controlled trial: (i) submission of two stool samples at baseline; (ii) detection of at least one soil-transmitted helminth egg in the samples; (iii) no major systemic illnesses as determined by a medical doctor from the Bulangshan township hospital; (iv) no known allergy to albendazole; (v) no de-worming treatment over the previous 6 months; (vi) no participation in other clinical trials; and (vii) residency in the study area for at least 1 year prior to enrollment, as assessed by a parental questionnaire.

11.3.2. Study design

The study was designed as a randomized, double-blind, placebo-controlled trial with multiple follow-ups. Triple-dose albendazole was administered in an attempt to achieve high cure rates, so that participating children have a fair chance of developing their physical fitness unaffected by ongoing intestinal helminth infections. The parasitological status of the children was assessed at baseline and at months 1, 4, and 6 after treatment. Given an estimated prevalence of 70% with any soil-transmitted helminth species and 50% loss to follow-up, we aimed at enrolling 250 children to submit stool samples at baseline in order to achieve a power of 80% at an alpha error of 5% for the detection of a $2.5 \text{ ml kg}^{-1} \text{ min}^{-1}$ difference in the maximum volume of oxygen that can be utilized during 1 min of exhaustive exercise (VO_2 max estimate) between the treatment and placebo groups.²³ Treatment allocation was determined by a statistician using block randomization with randomly varying block sizes of 2, 4, and 6 to ensure that both treatment arms had similar sample sizes.²⁴ Triple-dose non-flavored albendazole (400 mg) and placebo treatments (tablets matched in color, size, taste, and shape) were prepared by staff not involved in the field work, in sealed envelopes marked with unique

identifiers (IDs). Class lists were obtained from the schools and following the order of the list, each child who met the inclusion criteria was systematically assigned a random number from a list of random numbers generated from R.²⁵ The assigned random number for each child corresponded to the treatment number on the sealed envelope and thus, determined the type of treatment the child was allocated to.

11.3.3. Field and laboratory procedures

After written informed consent was obtained from parents/guardians of the children, stool containers, labeled with the name and IDs, were distributed. Containers, filled with fresh morning stool samples, were collected on the following day. From each child, two stool samples were collected over consecutive days.

Stool samples were transferred to a nearby laboratory and processed on the day of collection. Both the Kato-Katz and Baermann techniques were used.²⁶ In brief, for the Kato-Katz technique, a 41.7 mg template was used to prepare fecal thick smears. Thirty to 60 min after preparation, slides were read under a microscope at 100x magnification. The number of eggs was counted separately for *A. lumbricoides*, hookworm, and *T. trichiura*. For the Baermann technique, about 20 g of stool was placed on medical gauze in a glass funnel fitted with a rubber tube sealed by a clip, and filled with tap water. The whole set up was illuminated with artificial light directed at the bottom of the funnel for 2 hours. The lowest 50 mL of the liquid was then collected and centrifuged. The sediment was subjected to microscopic examination for the larvae of *Strongyloides stercoralis*. In addition, each stool sample was visually inspected for *Taenia* spp. proglottids. Taking the presence of *S. stercoralis* and *Taenia* spp. into account was necessary to control for potential confounders with regard to the primary outcome (i.e., physical fitness, to be reported elsewhere), in the form of additional intestinal helminth infections.

The assigned triple dose treatment (i.e., 3 x 400 mg albendazole (GlaxoSmithKline; London, UK) or 3 x placebo (Fagron; Barsbüttel, Germany)), was started on treatment day 1 with a single dose, with another dose administered every day until treatment day 3. Drug ingestion was observed by trained medical staff. Parasitological follow-up involved the same sample collection and analysis procedures. In addition, the socioeconomic status of the participants was assessed through a short

questionnaire asking for the education level of the children's parents and the main source of household income.

11.3.4. Statistical analysis

Data were entered into Excel version 2008 (Microsoft Corp.; Redmond, WA), double-checked, and merged into a single database for statistical analysis with STATA version 10.0 (STATA Corp.; College Station, TX). The randomization code was broken after data entry was completed and a series of internal consistency checks completed. A per-protocol analysis was carried out in an un-blinded manner.

The parasitological status of the study participants was described in terms of prevalence, infection intensity (geometric mean (GM)^{27,28} eggs per gram of stool (EPG)), and multiparasitism (concurrent infections with more than one helminth species). Re-infection patterns were established from changes in prevalence and infection intensity following treatment. Comparisons were between the albendazole and placebo treatment groups. Efficacy of triple-dose albendazole against placebo was measured in terms of cure and egg reduction rates. Cure rate is defined as the proportion of egg-positive individuals who became egg-negative after treatment. Egg reduction rate is calculated using the following equation: (GM EPG at baseline among infected – GM EPG at follow-up among those infected at baseline) / GM EPG at baseline among infected x 100.²⁷ All analyses were done on a per-species basis, except that of multiparasitism. Test statistics included chi-square (χ^2), Wilcoxon rank-sum, two-sample tests of proportions, and logistic regression, as appropriate.

11.3.5. Ethical considerations

The results reported here were obtained as part of a randomized, double-blind, placebo-controlled trial that has been registered with Current Controlled Trials (identifier: ISRCTN 25371788). The study protocol was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland). Ethical clearance was obtained from the ethics committee of Basel (EKBB, reference no. 144/11) and the Academic Board of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, P.R. China).

The village doctor, chief, and teachers of each village were briefed on the aims of the study and the planned procedures. With help from the teachers, the investigators

further explained the study to the children and their parents/ guardians. Written informed consent was obtained from parents/guardians and children assented orally. Parents/guardians were free to recall their children from the study anytime without further obligations, and children were informed that they could withdraw without negative impact for them or their family. Data were kept anonymous.

At study completion (i.e., after the 6-month follow-up), all children attending the five schools were given triple-dose albendazole (3 x 400 mg) treatment irrespective of their infection status, study participation, and treatment during the study (albendazole or placebo). In addition, children diagnosed with *S. stercoralis* during the study period were given a single dose of ivermectin (200 µg/kg). Brief education sessions on soil-transmitted helminth infections and prevention methods were conducted in all schools.

11.4. Results

11.4.1. Compliance and demographics of study participants

As shown in Figure 11.1, 229 children were interested to participate, and hence, they were assessed for eligibility. Eighteen children were excluded because they either did not submit two stool samples at baseline ($n = 16$) or were not infected with any soil-transmitted helminth species ($n = 2$). Randomization of treatments was done for 211 children and resulted in 106 subjects allocated to triple-dose albendazole and 105 children assigned to the placebo group. However, 2 and 6 children did not receive the full albendazole and placebo treatments, respectively, as they were sick or otherwise unavailable. Loss of children during the three follow-ups was small. Complete datasets were available for 194 children, 99 in the albendazole group, and 95 in the placebo group.

The age of the cohort at the start of the study ranged between 9 and 12 years. At baseline, the mean age of the albendazole group was 10.4 years (standard deviation (SD): 1.2 years), similar to the placebo group (mean 10.3 years, SD: 1.0 years; $P = 0.494$). The proportion of males in the albendazole and placebo groups was 46.5% and 50.5% respectively ($P = 0.571$). Socioeconomic conditions across the five villages were comparable, and no significant difference was detected between the albendazole and placebo groups. All children originated from families where the main source of income was agriculture, and the majority of their parents (56.2%) had not received formal education ($P = 0.108$).

11.4.2. Baseline helminth infection status and efficacy of triple-dose albendazole

The baseline prevalence of *T. trichiura*, *A. lumbricoides*, and hookworm infection was 94.5%, 93.3%, and 61.3%, respectively. Six cases of *S. stercoralis* (3.1%) and one case of *Taenia* spp. (0.5%) were detected. Most of the infected children harbored light *T. trichiura* infections (1–999 EPG; 86.3%) whereas 13.1% had infections of moderate intensity (1,000–9,999 EPG). Only 1 heavy infection ($\geq 10,000$ EPG) was detected. In contrast, more than half of the *A. lumbricoides*-infected children had moderate-intensity infections (5,000–49,999 EPG) and 27.6% had heavy infections ($\geq 50,000$ EPG). All but two hookworm infections were of light intensity (1–1,999 EPG). No significant differences in prevalence and intensity of infection were observed between the albendazole and placebo groups (Table 11.1). As illustrated in Figure 11.2, multiparasitism was common: 55.7% of the children were infected with all three common soil-transmitted helminth species concurrently, whereas a single-species infection was detected in only 6.7% of the children.

Cure rates of 96.7%, 91.5%, and 19.6% were achieved for hookworm, *A. lumbricoides*, and *T. trichiura* infections, respectively, with triple-dose albendazole. The treatment resulted in egg reduction rates of >99.9%, 99.1%, and 88.8% for *A. lumbricoides*, hookworm, and *T. trichiura* infections, respectively. Cure and egg reduction rates achieved with triple-dose albendazole were all significantly different from those observed after placebo administration (Table 11.1).

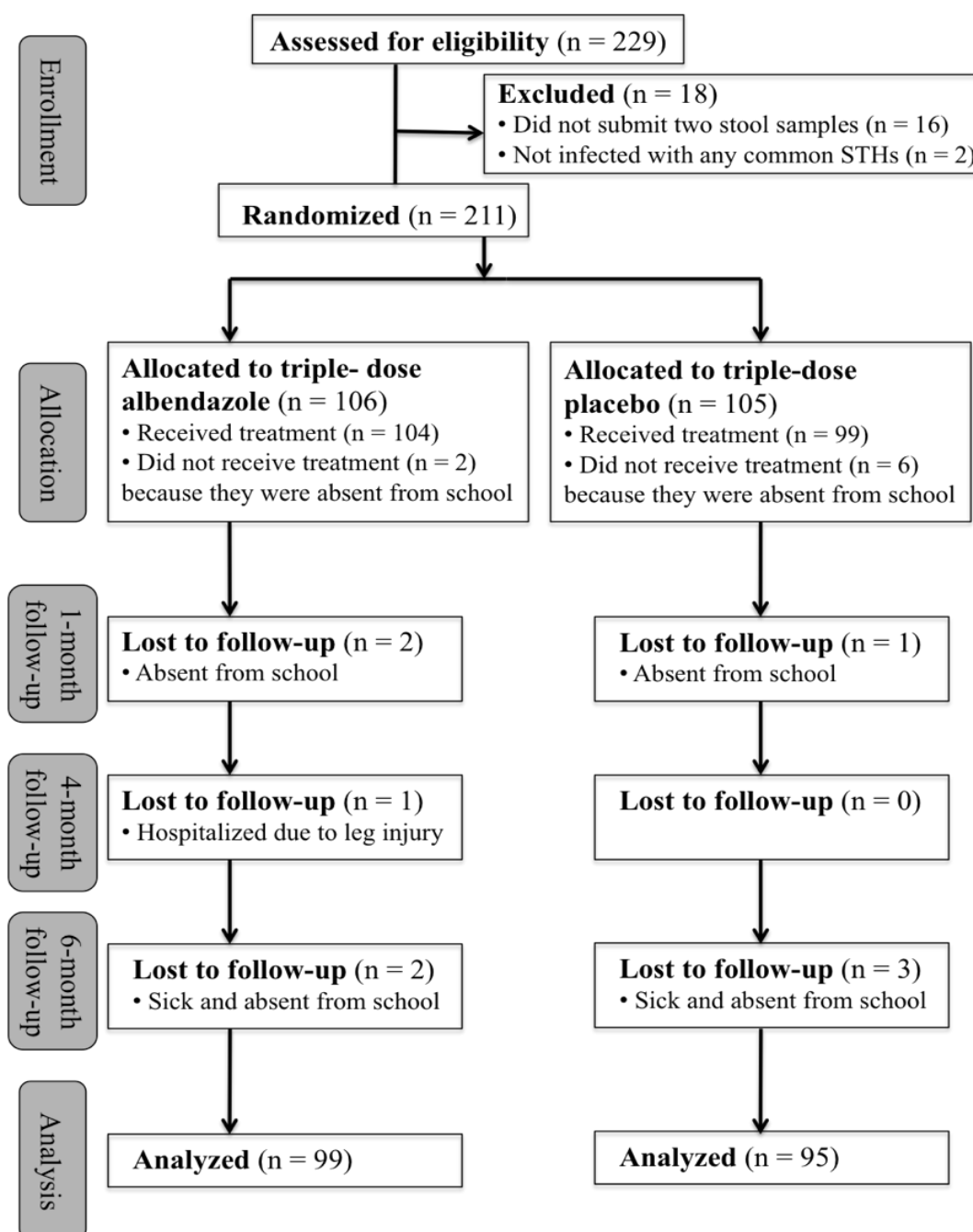


Figure 11.1. Trial flow diagram of a randomized controlled trial conducted in south-west Yunnan province, P.R. China, from October 2011 to May 2012, according to CONSORT guidelines.⁴⁹

Table 11.1. Prevalence, cure rates, infection intensities, and egg reduction rates of *A. lumbricoides*, *T. trichiura*, and hookworm at baseline and 1-month follow-up among 194 children from Yunnan, P.R. China, from October 2011 to May 2012, stratified by treatment groups.

	Baseline (Oct 2011)		1-month follow up (Dec 2011)		<i>P</i> – value ^c
	Triple-dose albendazole (N ^a = 99)	Placebo (N ^a = 95)	Triple-dose albendazole (N ^a = 99)	Placebo (N ^a = 95)	
	n ^b (%)	n ^b (%)	n ^b (%)	n ^b (%)	
<i>Ascaris lumbricoides</i>					
Prevalence	94 (95.0)	87 (91.6)	8 (8.1)	89 (93.7)	<0.001
New positives at 1-month follow-up	n.a.	n.a.	0	5	
Cure rate^d [% (95% confidence interval)]	n.a.	n.a.	91.5 (83.9 – 96.3)	3.4 (0.7 – 9.7)	
Difference between albendazole and placebo cure rates [% (95% confidence interval)]	n.a.	n.a.	88.1 (81.3 – 94.9)	Reference	<0.001
Infection intensity^e					
Mean EPG ^f (95% confidence interval)	15,850 (10,834 – 23,189)	19,101 (13,198 – 27,644)	1.3 (1.0 – 1.7)	21,001 (12,835 – 34,362)	<0.001
Light (1 – 4,999)	13 (13.1)	15 (15.8)	7 (7.1)	13 (13.7)	<0.001
Moderate (5,000 – 49,999)	54 (54.6)	49 (51.6)	1 (1.0)	45 (47.4)	
Heavy (≥ 50,000)	27 (27.3)	23 (24.2)	n.r.	31 (32.6)	
Egg reduction rate [% (95% confidence interval)^g]	n.a.	n.a.	>99.9 (96.2 – 100)	n.a.	
<i>Trichuris trichiura</i>					
Prevalence	92 (92.9)	91 (95.8)	74 (74.8)	92 (96.8)	<0.001
New positives at 1-month follow-up	n.a.	n.a.	0	1	
Cure rate^d [% (95% confidence interval)]	n.a.	n.a.	19.6 (12.0 – 29.1)	0	
Difference between albendazole and placebo cure rates [% (95% confidence interval)]	n.a.	n.a.	19.6 (11.5 – 27.7)	Reference	<0.001
Infection intensity^e					
Mean EPG ^f (95% confidence interval)	216.3 (160.2 – 292.0)	284.4 (207.2 – 390.4)	24.3 (16.2 – 36.3)	304.7 (227.4 – 408.3)	<0.001
Light (1 – 999)	82 (82.8)	76 (80.0)	73 (73.7)	73 (76.8)	<0.001
Moderate (1,000 – 9,999)	9 (9.1)	15 (15.8)	1 (1.0)	18 (19.0)	

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Heavy ($\geq 10,000$)	1 (1.0)	n.r.	n.r.	1 (1.1)	
Egg reduction rate [% (95% confidence interval)^g]	n.a.	n.a.	88.8 (80.9 – 94.7)	n.a.	
Hookworm					
Prevalence	60 (60.6)	59 (62.1)	2 (2.0)	57 (60.0)	<0.001
New positives at 1-month follow-up	n.a.	n.a.	0	6	
Cure rate^d [% (95% confidence interval)]	n.a.	n.a.	96.7 (88.5 – 99.6)	13.6 (6.0 – 25.0)	
Difference between albendazole and placebo cure rates [% (95% confidence interval)]	n.a.	n.a.	83.1 (73.3 – 92.9)	Reference	<0.001
Infection intensity^e					
Mean EPG ^f	130.4	121.5	1.2	62.2	<0.001
(95% confidence interval)	(93.3 – 182.2)	(86.7 – 170.3)	(0.9 – 1.5)	(37.7 – 102.6)	
Light (1 – 1,999)	59 (59.6)	58 (61.1)	2 (2.0)	57 (60.0)	<0.001
Moderate (2,000 – 3,999)	1 (1.0)	1 (1.0)	n.r.	n.r.	
Heavy ($\geq 4,000$)	n.r.	n.r.	n.r.	n.r.	
Egg reduction rate [% (95% confidence interval)^g]	n.a.	n.a.	99.1 (91.1 – 100)	48.8 (35.9 – 62.5)	<0.001

^a N: total sample size

^b n: number of infected individuals

^c P -values are calculated using χ^2 , two-sample test of proportions or Wilcoxon rank-sum test, as appropriate

^d Cure rate excludes newly infected at 1st follow-up

^e Stratified according to WHO guidelines

^f Geometric mean among those infected at baseline

^g Calculated by bootstrap resampling among those infected at baseline

n.a.: not applicable; n.r.: not represented

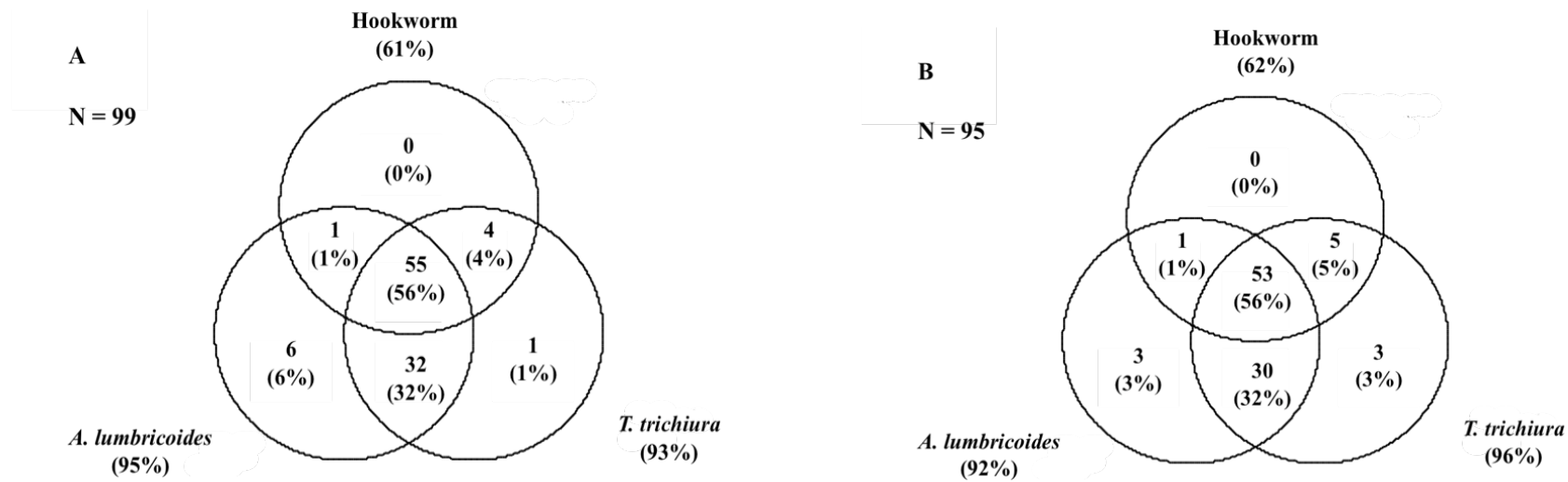


Figure 11.2. Extent of multiparasitism (common soil-transmitted helminths only) at baseline among 194 children from Yunnan, P.R. China, in October 2011, stratified by triple-dose albendazole (A) and placebo (B) treatment groups. No significant difference was detected between the groups ($P = 0.831$).

11.4.3. Re-infection patterns and dynamics

As shown in Figure 11.3, re-infection with *A. lumbricoides* was rapid. About three-quarter of the treated children were infected with this parasite 4 months after drug administration. Six months post-treatment, 83.8% of the children were infected with *A. lumbricoides*. Stratification of the *A. lumbricoides* infection intensity at the various follow-ups according to WHO guidelines showed a gradual shift in infection intensity in most of the children, from non-infection (91.9%) at the 1-month follow-up to moderate infection (52.5%) at the 6-month follow-up (Figure 11.4).

In the case of *T. trichiura* infections, prevalence remained high even at the 1-month post-treatment follow-up (74.8%); as a consequence, subsequent re-infection was slow, with 82.8% of the treated children harboring *T. trichiura* 6 months after treatment. For the treatment group, an 87% drop in the intensity of *T. trichiura* infection was detected at the 1-month follow-up, but infection intensity increased over time and was back to 30% of the baseline level at the 6-month follow-up (Figure 11.5).

Hookworm re-infection was minimal throughout the 6-month follow-up period as only 5.1% of the treated children were harboring hookworm at the end of the study. The hookworm infection intensity was reduced by more than 90% and maintained at this level across the various follow-ups.

When further stratified by baseline co-infection status, no significant difference in the prevalence of common soil-transmitted helminth infections at the follow-ups was detected among the treated children (results not shown). Among placebo recipients, no significant change in the prevalence and infection intensity levels of the three common soil-transmitted helminths at the various follow-ups was observed. Still, between the first and the second follow-up, a decrease of 8.4% in the hookworm prevalence was noted.

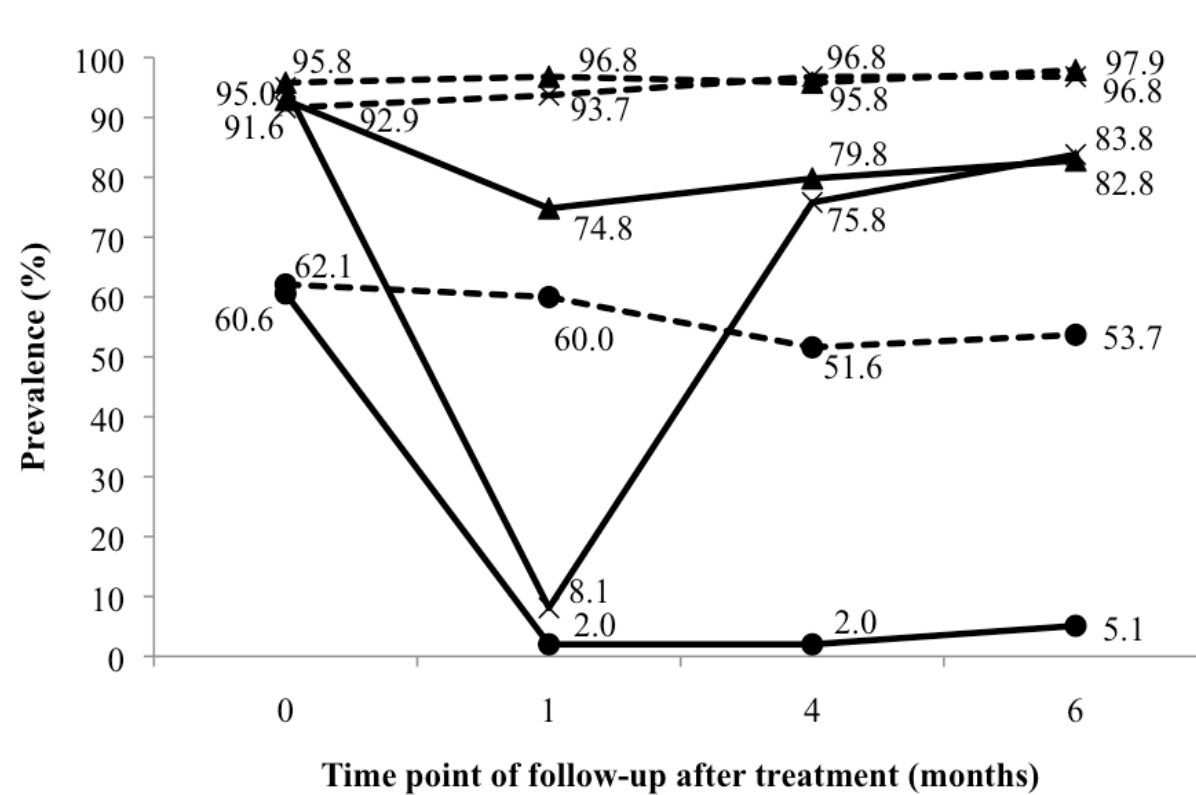


Figure 11.3. Prevalence of *A. lumbricoides* (x), *T. trichiura* (▲) and hookworm (●) among 194 children from Yunnan, P.R. China, in October 2011 – May 2012, stratified by triple-dose albendazole (solid lines) and placebo (dotted lines) treatment groups.

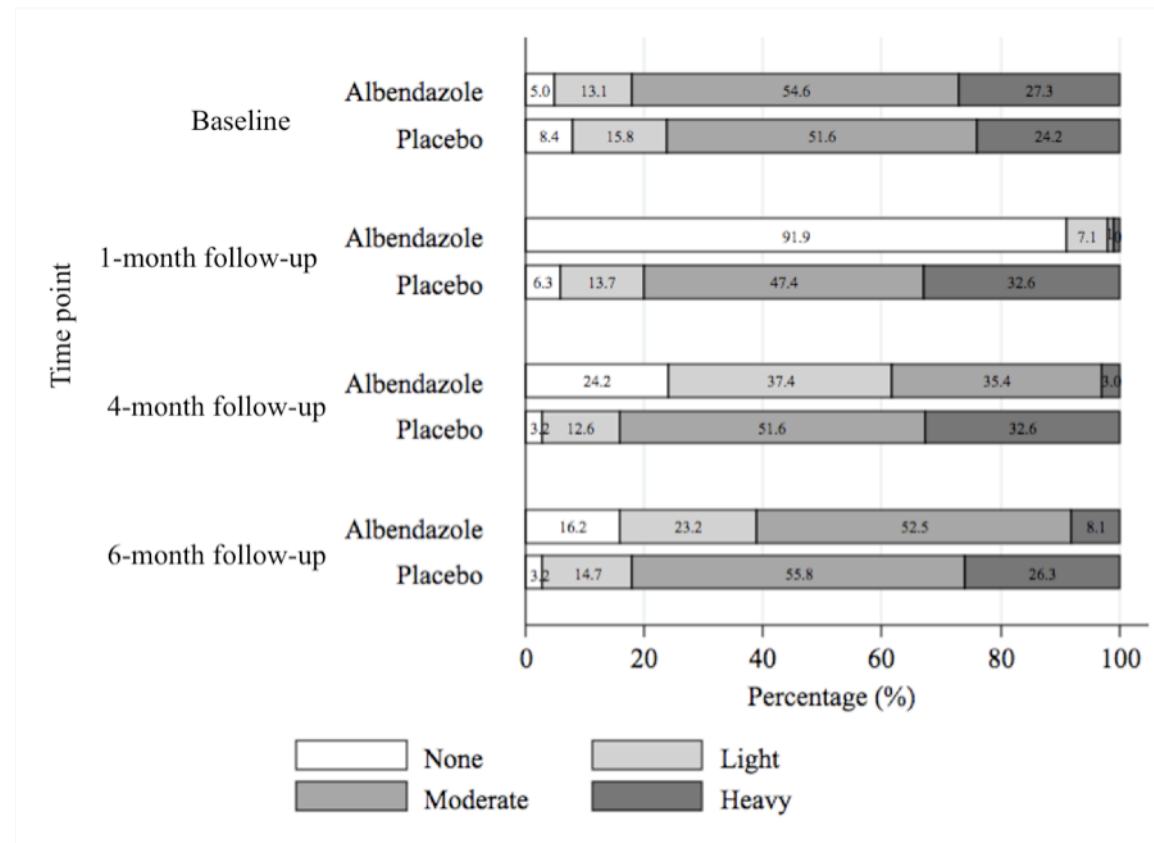


Figure 11.4. *A. lumbricoides* infection intensity levels, classified according to WHO guidelines, among 194 children from Yunnan, P.R. China, from October 2011 to May 2012.

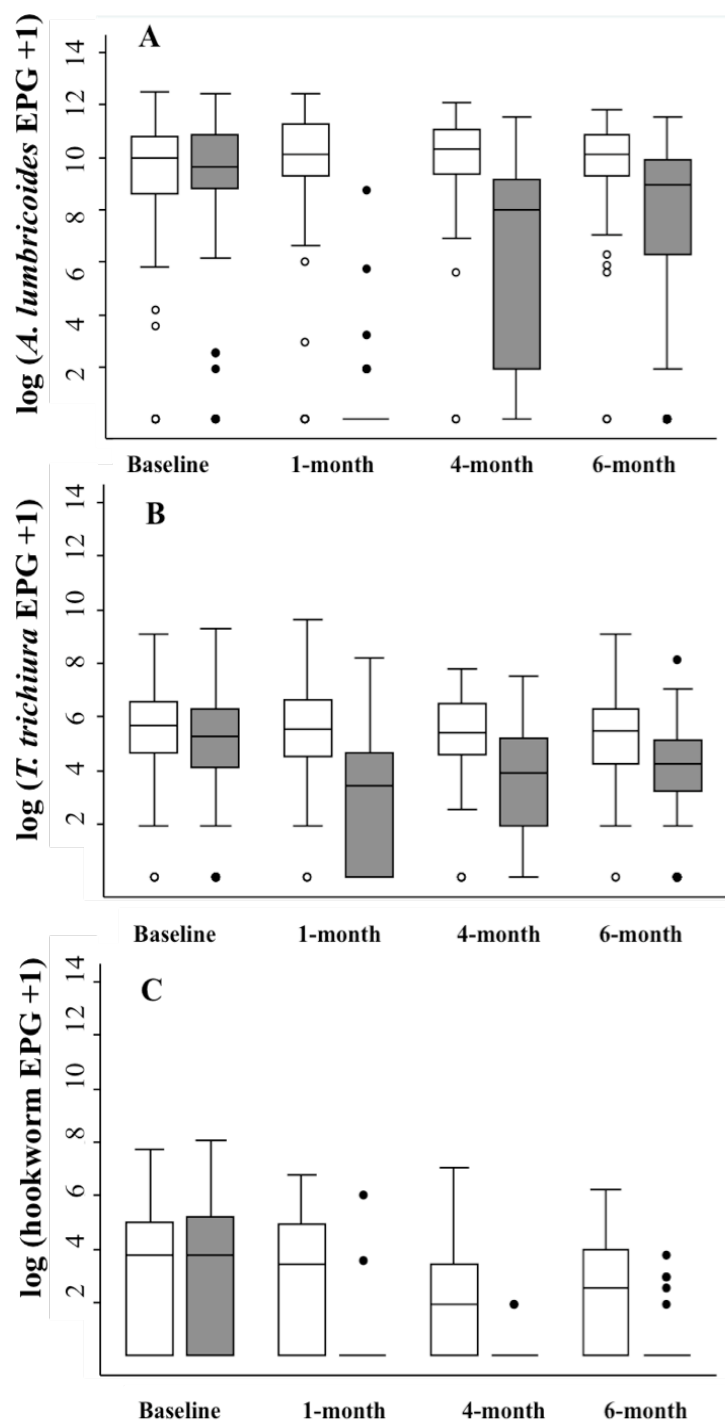


Figure 11.5. Infection intensities of *A. lumbricoides* (A), *T. trichiura* (B), and hookworm (C) at different time points among 194 children from Yunnan, P.R. China, from October 2011 to May 2012, stratified by triple-dose albendazole (grey bars) and placebo (white bars) treatment group. Middle line of box: median; upper end of box: 75th percentile; lower end of box: 25th percentile; upper whisker: 95th percentile; lower whisker: 5th percentile; dots: outliers.

11.4.4. Predictors of re-infection with *A. lumbricoides*

As re-infection was minimal for hookworm and the cure rate for *T. trichiura* was very low, odds of re-infections were not calculated for these two species. According to the results of the univariate logistic regression analyses (Supplementary table 11.1 under Appendix), children from Sandui and Laozhai had significantly higher odds to be re-infected with *A. lumbricoides* at the 4-month follow-up as compared to children from Kongkan (Sandui: odds ratio (OR) = 4.92, 95% confidence interval (CI): 1.24-19.57; Laozhai: OR = 3.74, 95% CI: 0.93-15.14). Likewise, the odds of *A. lumbricoides* re-infection of children living in Laozhai were higher (OR = 4.58, 95% CI: 0.91-23.14) at the 6-month follow-up as compared to children from Kongkan. Baseline infections with *A. lumbricoides* and *T. trichiura* increased the odds of re-infection with *A. lumbricoides* by 2.18 and 2.54 times, respectively, at the 4-month follow-up and by 1.32 and 2.23 times, respectively, at the 6-month follow-up. However, these risk increases lacked statistical significance. Heavy baseline *A. lumbricoides* infection intensity was a positive but, for the current sample size, insignificant predictor of *A. lumbricoides* re-infection (OR = 8.33 at the 4-month follow-up, and OR = 6.50 at the 6-month follow-up). Age and sex showed no significant associations with *A. lumbricoides* re-infection.

In the multivariate logistic regression analyses, village location remained a significant predictor of *A. lumbricoides* re-infection. At the 4-month follow-up, the odds of re-infection of children living in Sandui were increased by a factor of 9.71 (95% CI: 1.72-54.61) as compared to that of children living in Kongkan.

11.5. Discussion

Few studies have assessed post-treatment re-infection patterns with soil-transmitted helminths as rigorously as reported here, with the study integrated into a randomized controlled trial. According to the national parasitological survey conducted in P.R. China from 2001–2004²⁹, soil-transmitted helminth infections were most prevalent in the provinces of Guizhou, Hainan, and Hunan, and Guangxi Zhuang Autonomous region. National prevalence rates were 12.7%, 6.1%, and 4.6% for *A. lumbricoides*, hookworm, and *T. trichiura*, respectively. However, cross-sectional studies conducted recently showed important variations in the prevalence and intensity of soil-transmitted helminth infections in different geographical regions across P.R. China.^{20,30} Among

school-aged children, rural areas in Hainan province and Guangxi Zhuang Autonomous region reported prevalences of 18.5%, 14.7%, and 11.2% for *A. lumbricoides*, hookworm, and *T. trichiura*, respectively, with 16.7% of the infections being of moderate or heavy intensity.³¹ In another rural part of Yunnan province, prevalences of 35.7%, 5.4%, and 2.7% were reported for hookworm, *T. trichiura* and, *A. lumbricoides*, respectively.³² The baseline prevalence and intensity of soil-transmitted helminth infections documented here are among the highest ever reported from P.R. China. However, they are comparable to reports by Steinmann and co-workers³³ from other Bulang communities characterized by similar socio-ecological contexts where multiple species helminth infection was also common.^{11,20-22} Taken together, these data emphasize that, although P.R. China has witnessed huge economic and social improvements over the past 30 years, hotspots of soil-transmitted helminthiasis and other neglected tropical diseases persist³⁴, with ethnic minority groups particularly vulnerable and thus, less benefiting from the country's development.^{35,36}

Post-treatment re-infection patterns of soil-transmitted helminths are largely dependent on the degree of endemicity within the community.^{37,38} In the current high endemicity area of P.R. China, follow-ups at months 1, 4, and 6 after administration of triple-dose albendazole or placebo revealed that re-infection with *A. lumbricoides* was rapid, both in terms of prevalence and intensity. Indeed, the prevalence of *A. lumbricoides* reached 80% of the pretreatment prevalence 4 months after treatment. Our findings corroborate recent results from a systematic review and meta-analysis by Jia and colleagues.¹⁶ The high egg production and long survival of the infective stage (the ova) of *A. lumbricoides* in the environment could explain the quick re-infection wherever permissive hygiene and behavioral conditions coincide and no measures to prevent re-infection have been implemented.⁶

Recent publications have highlighted the low efficacy of albendazole against *T. trichiura* infections.³⁹⁻⁴² A single oral dose of 400 mg of albendazole was reported to give a cure rate of 28% in a meta-analysis.¹⁵ Generally, the previously reported cure rates, particularly following triple-dose treatment, were considerably higher than the one reported in this trial. Indeed, the cure rate of only 19.6% for *T. trichiura* as observed in our trial is much lower than that reported from a previous trial with triple-dose albendazole treatment in the same area (56%).³³ It is difficult to explain these observations as sampling and diagnostic efforts were comparable and the same field team

was involved in both trials. It should be noted, however that from a public health point of view, the egg reduction rate rather than the cure rate should be considered when determining anthelmintic drug efficacy.⁴³ Indeed, the egg reduction rate more accurately reflects the reduction in morbidity, as it is associated with decreases in soil-transmitted helminths infection intensity. We observe an egg reduction rate of 88.8% for *T. trichiura*, which is reasonably high. However, the very low cure rate reported in our trial is of concern and underscores the pressing need for the development of novel anthelmintics and the study of drug resistance. We were interested in re-infection patterns after treatment, but this proved to be difficult for *T. trichiura* as only few of the infections completely cleared even after triple-dose administration.^{44,45}

Interestingly, the prevalence of hookworm was greatly reduced by triple-dose albendazole and the resulting low prevalence was maintained over the 6-month follow-up period, whereas prevalences of *A. lumbricoides* and *T. trichiura* were steadily increasing. Re-infection with hookworm is known to be slower compared to other common soil-transmitted helminth species as the reproductive number (R_0) of hookworm is the lowest among them,⁶ and the time between the 1- and the 4-month follow-ups (December–March) were cold and dry, conditions known to be unsuitable for the development and survival of hookworm larvae.^{6,46} The latter could also explain the slight decrease in the hookworm prevalence and infection intensity noted in the placebo group during the second follow-up. It follows that deworming should be deployed at the start of the dry season (co-incidentally coinciding with the return of the students to school after the summer break) in order to maximize the time they can expect to remain free from re-infection.

In terms of *S. stercoralis* infection, a prevalence of 3.1% detected in this school-based trial is considerably lower than what was observed in a previous community-based study in the same region (11.7%),⁴⁷ which might again point to the fact that *S. stercoralis* prevalence increases with age⁴⁸. However, the few cases present were observed to be of higher intensity, as inferred from the larger number of first-stage (L_1) larvae present, than noted in former work. Unfortunately, quantification of *S. stercoralis* infections to further substantiate such a claim is currently not possible.

We conclude that the observed re-infection patterns with soil-transmitted helminths after triple-dose albendazole re-emphasize the need for control programs that go beyond preventive chemotherapy, particularly for ascariasis and trichuriasis. In areas

of such intense transmission, even the stated goal of preventive chemotherapy, namely the prevention of morbidity through the depression of infection intensity levels, might be unattainable, particularly in the case of *A. lumbricoides*. Of note, the studied population is hitherto not covered by regular deworming activities. The need for a more effective drug or combination therapy against *T. trichiura* is further highlighted.

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11.6. References

1. Brooker S, 2010. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers-a review. *Int J Parasitol* 40: 1137-1144.
2. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Bas-Öez M-G, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabè E, Bhalla K, Bhandari B, Bikbov B, Abdulhak AB, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT-A, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, Leo DD, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fèvre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FGR, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gonzalez-Medina D, Gosselin R, Grainger R, Grant B, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo J-P, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Laden F, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Levinson D, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer A-C, Miglioli V, Miller M, Miller TR, Mitchell PB, Mock C, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KMV, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA, Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiah KD, Ranganathan D, Razavi H, Regan M, Rehm JrT, Rein DB, Remuzzi

- G, Richardson K, Rivara FP, Roberts T, Robinson C, De Le  n FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJC, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsens T, Tsilimbaris MK, Ubeda C, Undurraga EA, van der Werf MJ, van Os J, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiebe N, Wiersma ST, Wilkinson JD, Williams HC, Williams SRM, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh P-H, Zaidi AKM, Zheng Z-J, Zonies D, Lopez AD, 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380: 2197-2223.
3. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Ehrlich Sachs E, Sachs JD, Savioli L, 2007. Control of neglected tropical diseases. *N Engl J Med* 357: 1018-1027.
4. Utzinger J, Becker SL, Knopp S, Blum J, Neumayr AL, Keiser J, Hatz CF, 2012. Neglected tropical diseases: diagnosis, clinical management, treatment and control. *Swiss Med Wkly* 142: w13727.
5. Cox FEG, 2002. History of human parasitology. *Clin Microbiol Rev* 15: 595-612.
6. Brooker S, Clements ACA, Bundy DAP, 2006. Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol* 62: 221-261.
7. Hotez PJ, Bundy DAP, Beegle K, Brooker S, Drake L, de Silva N, Montresor A, Engels D, Jukes M, Chitsulo L, Chow J, Laxminarayan R, Michaud C, Bethony J, Correa-Oliveira R, Xiao SH, Fenwick A, Savioli L, 2006. Helminth infections: soil-transmitted helminth infections and schistosomiasis. Washington: World Bank.
8. Crompton DWT, Nesheim MC, 2002. Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr* 22: 35-59.
9. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J, 2008. Helminth infections: the great neglected tropical diseases. *J Clin Invest* 118: 1311-1321.
10. Nga TT, Winichagoon P, Dijkhuizen MA, Khan NC, Wasantwisut E, Wieringa FT, 2011. Decreased parasite load and improved cognitive outcomes caused by deworming and consumption of multi-micronutrient fortified biscuits in rural Vietnamese schoolchildren. *Am J Trop Med Hyg* 85: 333-340.
11. Yap P, Du ZW, Chen R, Zhang LP, Wu FW, Wang J, Wang XZ, Zhou H, Zhou XN, Utzinger J, Steinmann P, 2012. Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. *Parasit Vectors* 5: 50.

12. WHO, 2006. Preventive chemotherapy in human helminthiasis: coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme managers. Geneva: World Health Organization.
13. WHO, 2012. Eliminating soil-transmitted helminthiases as a public health problem in children. Geneva: World Health Organization.
14. Prichard RK, Basáñez MG, Boatin BA, McCarthy JS, Garcia HH, Yang GJ, Sripa B, Lustigman S, 2012. A research agenda for helminth diseases of humans: intervention for control and elimination. *PLoS Negl Trop Dis* 6: e1549.
15. Keiser J, Utzinger J, 2008. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* 299: 1937-1948.
16. Jia TW, Melville S, Utzinger J, King CH, Zhou XN, 2012. Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 6: e1621.
17. Gabrielli AF, Montresor A, Chitsulo L, Engels D, Savioli L, 2011. Preventive chemotherapy in human helminthiasis: theoretical and operational aspects. *Trans R Soc Trop Med Hyg* 105: 683-693.
18. Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P, 2012. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin and school performance. *Cochrane Database Syst Rev* 11: CD000371.
19. Humphries D, Nguyen S, Boakye D, Wilson M, Cappello M, 2012. The promise and pitfalls of mass drug administration to control intestinal helminth infections. *Curr Opin Infect Dis* 25: 584-589.
20. Steinmann P, Du ZW, Wang LB, Wang XZ, Jiang JY, Li LH, Marti H, Zhou XN, Utzinger J, 2008. Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. *Am J Trop Med Hyg* 78: 760-769.
21. Steinmann P, Zhou XN, Du ZW, Jiang JY, Xiao SH, Wu ZX, Zhou H, Utzinger J, 2008. Tribendimidine and albendazole for treating soil-transmitted helminths, *Strongyloides stercoralis* and *Taenia* spp.: open-label randomized trial. *PLoS Negl Trop Dis* 2: e322.
22. Ziegelbauer K, Steinmann P, Zhou H, Du ZW, Jiang JY, Fürst T, Jia TW, Zhou XN, Utzinger J, 2010. Self-rated quality of life and school performance in relation to helminth infections: case study from Yunnan, People's Republic of China. *Parasit Vectors* 3: 61.
23. Eng J, 2003. Sample size estimation: how many individuals should be studied? *Radiology* 227: 309-313.
24. Kang M, Ragan BG, Park JH, 2008. Issues in outcomes research: an overview of randomization techniques for clinical trials. *J Athl Train* 43: 215-221.

25. R Development Core Team, 2010. R: a language and environment for statistical analysis. Vienna: R Development Core Team.
26. Yap P, Fürst T, Müller I, Kriemler S, Utzinger J, Steinmann P, 2012. Determining soil-transmitted helminth infection status and physical fitness of school-aged children. *J Vis Exp* 66: 3966.
27. Montresor A, Crompton DWT, Hall A, Bundy DAP, 1998. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. Geneva: World Health Organization.
28. Danso-Appiah A, Garner P, Olliaro PL, Utzinger J, 2009. Treatment of urinary schistosomiasis: methodological issues and research needs identified through a Cochrane systematic review. *Parasitology* 136: 1837-1849.
29. Coordinating Office of the National Survey on the Important Human Parasitic Diseases, 2005. [A national survey on current status of the important parasitic diseases in human population]. *Chin J Parasitol Parasit Dis* 23: 332-340 (in Chinese).
30. Balen J, Raso G, Li YS, Zhao ZY, Yuan LP, Williams GM, Luo XS, Shi MZ, Yu XL, Utzinger J, McManus DP, 2011. Risk factors for helminth infections in a rural and a peri-urban setting of the Dongting Lake area, People's Republic of China. *Int J Parasitol* 41: 1165-1173.
31. Shang Y, Tang LH, Zhou SS, Chen YD, Yang YC, Lin SX, 2010. Stunting and soil-transmitted-helminth infections among school-age pupils in rural areas of southern China. *Parasit Vectors* 3: 97.
32. Mofid LS, Bickle Q, Jiang JY, Du ZW, Patrick E, 2011. Soil-transmitted helminthiasis in rural south-west China: prevalence, intensity and risk factor analysis. *Southeast Asian J Trop Med Public Health* 42: 513-526.
33. Steinmann P, Utzinger J, Du ZW, Jiang JY, Chen JX, Hattendorf J, Zhou H, Zhou XN, 2011. Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp.: a randomized controlled trial. *PLoS One* 6: e25003.
34. Hotez PJ, 2012. Engaging a rising china through neglected tropical diseases. *PLoS Negl Trop Dis* 6: e1599.
35. Utzinger J, Bergquist R, Olveda R, Zhou XN, 2010. Important helminth infections in Southeast Asia diversity, potential for control and prospects for elimination. *Adv Parasitol* 72: 1-30.
36. Hotez PJ, Ehrenberg JP, 2010. Escalating the global fight against neglected tropical diseases through interventions in the Asia Pacific region. *Adv Parasitol* 72: 31-53.
37. Albonico M, Smith PG, Ercole E, Hall A, Chwaya HM, Alawi KS, Savioli L, 1995. Rate of reinfection with intestinal nematodes after treatment of children

- p>with mebendazole or albendazole in a highly endemic area.
- Trans R Soc Trop Med Hyg*
- 89: 538-541.
38. Saathoff E, Olsen A, Kvalsvig JD, Appleton CC, 2004. Patterns of geohelminth infection, impact of albendazole treatment and re-infection after treatment in schoolchildren from rural KwaZulu-Natal/South-Africa. *BMC Infect Dis* 4: 27.
39. Vercruysse J, Behnke JM, Albonico M, Ame SM, Angebault C, Bethony JM, Engels D, Guillard B, Nguyen TV, Kang G, Kattula D, Kotze AC, McCarthy JS, Mekonnen Z, Montresor A, Periago MV, Sumo L, Tchuente LA, Dang TC, Zeynudin A, Levecke B, 2011. Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. *PLoS Negl Trop Dis* 5: e948.
40. Namwanje H, Kabatereine NB, Olsen A, 2011. Efficacy of single and double doses of albendazole and mebendazole alone and in combination in the treatment of *Trichuris trichiura* in school-age children in Uganda. *Trans R Soc Trop Med Hyg* 105: 586-590.
41. Speich B, Ame SM, Ali SM, Alles R, Hattendorf J, Utzinger J, Albonico M, Keiser J, 2012. Efficacy and safety of nitazoxanide, albendazole, and nitazoxanide-albendazole against *Trichuris trichiura* infection: a randomized controlled trial. *PLoS Negl Trop Dis* 6: e1685.
42. Geary TG, 2012. Are new anthelmintics needed to eliminate human helminthiasis? *Curr Opin Infect Dis* 25: 709-717.
43. Montresor A, 2011. Cure rate is not a valid indicator for assessing drug efficacy and impact of preventive chemotherapy interventions against schistosomiasis and soil-transmitted helminthiasis. *Trans R Soc Trop Med Hyg* 105: 361-363.
44. Keiser J, Utzinger J, 2010. The drugs we have and the drugs we need against major helminth infections. *Adv Parasitol* 73: 197-230.
45. Geary TG, Woo K, McCarthy JS, Mackenzie CD, Horton J, Prichard RK, de Silva NR, Olliaro PL, Lazdins-Helds JK, Engels DA, Bundy DA, 2010. Unresolved issues in anthelmintic pharmacology for helminthiasis of humans. *Int J Parasitol* 40: 1-13.
46. CDC, 2013. Hookworm: biology. Available at: <http://www.cdc.gov/parasites/hookworm/biology.html>; accessed February 21, 2013.
47. Steinmann P, Zhou XN, Du ZW, Jiang JY, Wang LB, Wang XZ, Li LH, Marti H, Utzinger J, 2007. Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and comparison of diagnostic methods. *PLoS Negl Trop Dis* 1: e75.
48. Becker SL, Sieto B, Silue KD, Adjossan L, Kone S, Hatz C, Kern WV, N'Goran EK, Utzinger J, 2011. Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Negl Trop Dis* 5: e1292.

49. Schulz KF, Altman DG, Moher D, 2010. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340: c332.

11.7 Appendix

Supplementary table 11.1. Univariate logistic regression analyses of the odds of re-infection with *A. lumbricoides* at 4- and 6-month follow-up among the 99 triple-dose albendazole-treated children from Yunnan, P.R. China, from October 2011 to May 2012.

	4-month follow-up		6-month follow-up	
	n_r/n_t^a (%)	OR (95% CI) ^b	n_r/n_t^a (%)	OR (95% CI) ^b
Baseline infection with <i>Ascaris lumbricoides</i>				
Non-infected	3/5 (60.0)	1.00	4/5 (80.0)	1.00
Infected	72/94 (76.6)	2.18 (0.34 – 13.90)	79/94 (84.0)	1.32 (0.14 – 12.61)
Baseline infection with <i>Trichuris trichiura</i>				
Non-infected	4/7 (57.1)	1.00	5/7 (71.4)	1.00
Infected	71/92 (77.2)	2.54 (0.53 – 12.24)	78/92 (84.8)	2.23 (0.39 – 12.64)
Baseline infection with hookworm				
Non-infected	30/39 (76.9)	1.00	34/39 (87.2)	1.00
Infected	45/60 (75.0)	0.90 (0.35 – 2.32)	49/60 (81.7)	0.66 (0.21 – 2.06)
Baseline infection intensity of <i>Ascaris lumbricoides</i>				
Non-infected	3/5 (60.0)	1.00	4/5 (80.0)	1.00
Light	9/13 (69.2)	1.50 (0.18 – 12.78)	10/13 (76.9)	0.83 (0.07 – 10.60)
Moderate	38/54 (70.4)	1.58 (0.24 – 10.40)	43/54 (79.6)	0.98 (0.10 – 9.64)
Heavy	25/27 (92.6)	8.33 (0.84 – 82.86)	26/27 (96.3)	6.50 (0.34 – 126.06)
Baseline level of multiparasitism				
Single species	5/7 (71.4)	1.00	6/7 (85.7)	1.00
Double species	27/37 (73.0)	1.08 (0.18 – 6.49)	31/37 (83.8)	0.86 (0.09 – 8.51)
Triple species	43/55 (78.2)	1.43 (0.25 – 8.33)	46/55 (83.6)	0.85 (0.09 – 7.96)
Age^c	n.a.	0.81 (0.55 – 1.19)	n.a.	0.84 (0.55 – 1.29)
Sex				
Male	36/46 (78.3)	1.00	37/46 (80.4)	1.00
Female	39/53 (73.6)	0.77 (0.31 – 1.96)	46/53 (86.8)	1.60 (0.54 – 4.70)
Village				
Kongkan	22/35 (62.9)	1.00	24/35 (68.6)	1.00
Sandui	25/28 (89.3)	4.92 (1.24 – 19.57) ^d	28/28 (100.0)	n.a. ^e
Laozhai	19/22 (86.4)	3.74 (0.93 – 15.14) ^d	20/22 (90.9)	4.58 ^c (0.91 – 23.14) ^c
Laonandong	4/6 (66.7)	1.18 (0.19 – 7.37)	5/6 (83.3)	2.29 (0.24 – 22.02)
Mannuo	5/8 (62.5)	0.98 (0.20 – 4.82)	6/8 (75.0)	1.38 (0.24 – 7.93)

^a n_r/n_t = number of individuals re-infected/total number of individuals in stratum

^b All values are odds ratio, with 95% confidence intervals in brackets, and all *P* – values are calculated from the likelihood ratio test

^c Age in years at the point of follow-up as a numeric variable

^d *P*–value <0.05

^e All children in Sandui were re-infected during the final follow-up

n.a.: not applicable

12. Influence of nutrition on re-infection with soil-transmitted helminths: a systematic review

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12.1. Abstract

Objective To conduct a systematic review examining the influence of nutrition on re-infection with soil-transmitted helminths in humans. Emphasis is placed on the use of nutritional supplementation, alongside anthelmintic treatment, to prevent re-infection with soil-transmitted helminths.

Methods We searched 8 major electronic databases from inception to 31 July 2013, with no restriction of language or type of publication. For studies that met our inclusion criteria, we extracted information on the soil-transmitted helminth species, nutritional supplementation, and anthelmintic treatment. Outcomes were presented in a summary of findings (SoF) table. An evidence profile (EP) was generated by rating the evidence quality of the identified studies according to the GRADE system.

Results Fifteen studies met our inclusion criteria; 8 randomised controlled trials and 7 prospective cohort studies. Data on *Ascaris lumbricoides* were available from all studies, whereas 7 and 6 studies additionally contained data on *Trichuris trichiura* and hookworm, respectively. Positive effects of nutritional supplementation or the host's natural nutritional status on re-infection with soil-transmitted helminths were reported in 14 studies, while negative effects were documented in 6 studies. In terms of quality, a high, low and very low quality rating was assigned to the evidence from 4, 6 and 5 studies, respectively.

Conclusions Our findings suggest that the current evidence-base is weak, precluding guidelines on nutrition management as a supplementary tool to preventive chemotherapy targeting soil-transmitted helminthiasis. Moreover, several epidemiological, immunological and methodological issues, which should be considered when designing future studies, have been identified.

Keywords soil-transmitted helminths, nutrition, anthelmintic treatment, re-infection, systematic review

12.2. Introduction

There is a Chinese proverb that goes “Let food be medicine” (translation of “以食为疗”). The traditional belief that inspired this saying is that in addition to nutritional value, food has medicinal properties too. Today, it is well established that the consumption of appropriate nutrients is critical to build up one’s immune defense against infectious pathogens (Kau *et al.* 2011). Indeed, it has been shown that undernutrition increases the general susceptibility of an individual to viral, bacterial and parasitic infections (Carr *et al.* 1999; Gershwin *et al.* 2000; Ing *et al.* 2000; Katona *et al.* 2008; Maggini *et al.* 2010), while infections negatively impact on the nutritional status, resulting in a vicious cycle of undernutrition and infection (Scrimshaw *et al.* 1959, 1997; Keusch 2003).

Roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) are collectively termed soil-transmitted helminths (Cox 2002). Soil-transmitted helminthiasis are the most prevalent neglected tropical diseases; over 5 billion people are at risk and more than 1 billion people are currently infected (Bethony *et al.* 2006; Hotez *et al.* 2007; Brooker 2010; Pullan *et al.* 2012). The global burden attributable to these intestinal nematode infections is estimated at 5.2 million disability-adjusted life years (DALYs) (Murray *et al.* 2012). Infections are concentrated in impoverished communities in tropical and sub-tropical regions of sub-Saharan Africa, Asia and Latin America, where poor personal hygiene practices are common and the provision of sanitation and clean water is deficient (Brooker *et al.* 2006; Pullan *et al.* 2012; Chammartin *et al.* 2013). The World Health Organization (WHO) advocates preventive chemotherapy, the regular administration of anthelmintic drugs to at-risk populations, to control the morbidity due to soil-transmitted helminthiasis (Utzinger *et al.* 2004; WHO 2012). However, re-infection occurs rapidly, especially in the absence of targeted health education and measures to improve sanitation and water supply (Hall 2007; Jia *et al.* 2012; Ziegelbauer *et al.* 2012).

In the context of the systematic review presented here, undernutrition is defined as the outcome of insufficient intake, or failure of the body to absorb, one or more essential macro- and micronutrients (Gershwin *et al.* 2000). According to the Food and Agriculture Organization (FAO) of the United Nations (UN), the global number of undernourished people stood at approximately 870 million in the years 2010 to 2012. The majority of

them lived in sub-Saharan Africa, Southern Asia and the Caribbean (FAO 2012). Given the geographical overlap with areas where the highest prevalences of soil-transmitted helminth infections are found, it is not surprising that a considerable fraction of undernourished people are simultaneously infected with soil-transmitted helminths. As illustrated in Figure 12.1, an individual harbouring soil-transmitted helminths might experience anaemia, physiological damage to the gastrointestinal system and other symptoms, which in turn can exacerbate nutritional deficiencies. It is commonly accepted that this could ultimately lead to impaired growth and cognitive development (Stephenson *et al.* 2000; Crompton *et al.* 2002; Zhou *et al.* 2007; Mupfasoni *et al.* 2009; Hall *et al.* 2012). However, findings from studies pertaining to undernutrition and the susceptibility to soil-transmitted helminthiasis are inconclusive, and the true cause-effect relationship, as well as the magnitude of any causal link, remains to be investigated (Koski *et al.* 2001).

To address both undernutrition and soil-transmitted helminthiasis, a dual intervention approach has been proposed (Katona *et al.* 2008). Nutritional supplementation is commonly employed to address undernutrition and has been proven to be effective in reducing nutritional deficiencies (Stratton *et al.* 2010). However, nutrition management, alongside anthelmintic treatments and with an aim to reduce re-infection with soil-transmitted helminths, remains controversial, and its practical significance and public health potential have yet to be fully explored (Koski *et al.* 2001). Given that one of the Millennium Development Goals (MDGs) is to “halve, between 1990 and 2015, the proportion of people who suffer from hunger” (UN 2010), the role of nutrition management on the re-infection with soil-transmitted helminths should be investigated. This systematic review summarises the available evidence on influence of nutrition on re-infection with soil-transmitted helminths in humans, with a focus on the use of nutritional supplementation, provided together with anthelmintic drugs, to reduce re-infection.

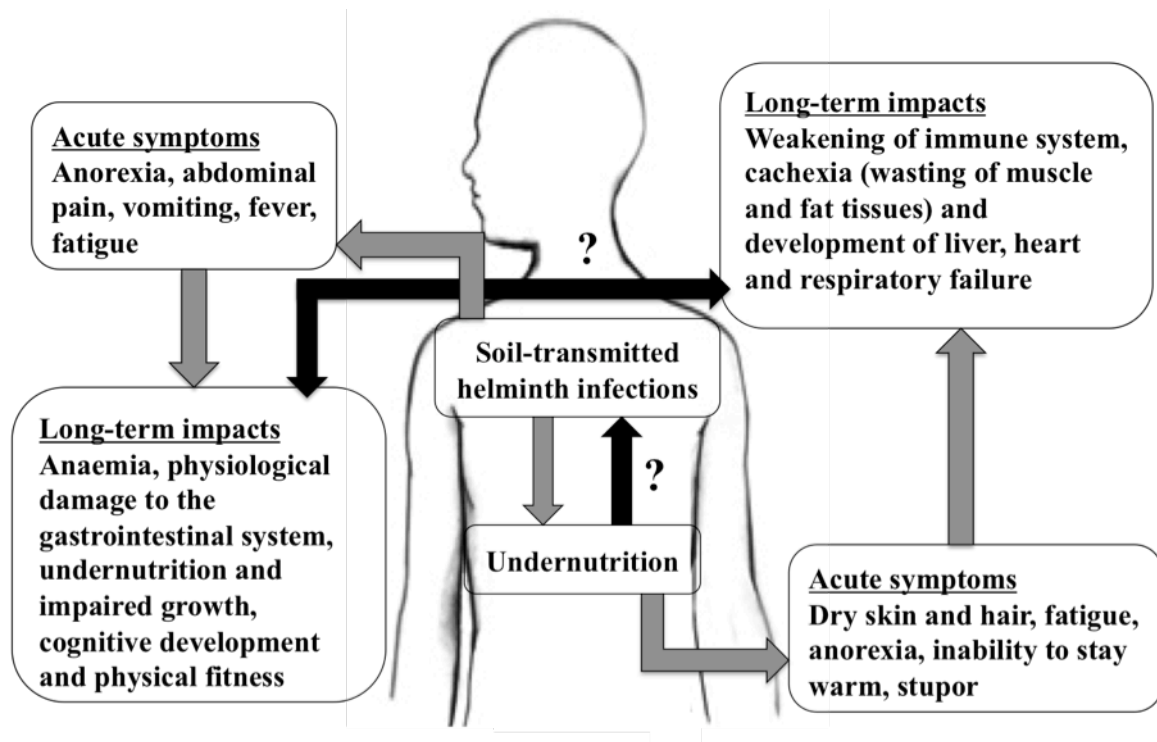


Figure 12.1. Conceptual framework underpinning this systematic review. The black arrows indicate research gaps in the understanding of the interactions between soil-transmitted helminth infections and undernutrition.

12.3. Methods

12.3.1. Criteria for considering studies for this review

Types of studies

We included randomised controlled trials and prospective cohort studies.

Types of outcome measures

Primary outcomes for single and concurrent infections with soil-transmitted helminths were: (i) re-infection rate described as (changes in) prevalence (%); and (ii) (changes in) infection intensity in terms of (changes in) eggs per gram of stool (EPG). Secondary outcomes were: (i) immune responses to nutritional supplementation and relevant for soil-transmitted helminth infections; and (ii) other health benefits of nutritional supplementation. Reported detrimental effects of nutritional supplementation in study participants were recorded as adverse outcomes.

Types of comparisons

The following comparisons were included in this review: (i) macro-nutrient supplementation *versus* placebo; (ii) multi-micronutrient supplementation *versus* placebo; (iii) single micronutrient supplementation *versus* placebo; and (iv) undernourished hosts *versus* well-nourished hosts (in their natural states).

Exclusion criteria

Cross-sectional or case-control studies that compared the nutrition status or growth of infected *versus* non-infected hosts (in their natural states or treated with anthelmintics) were excluded for their lack of potential to demonstrate causality. Reviews and studies that focused on interactions between malnutrition and immunity in general, with no specific reference to soil-transmitted helminths, were excluded. Lastly, case reports or case series were also excluded.

12.3.2. Search methods for identification of studies

We searched 8 readily available electronic databases: (i) PubMed/Medline; (ii) Embase; (iii) Cochrane Library; (iv) Cochrane Central Register of Controlled Trials (CENTRAL); (v) Virtual Health Library (VHL); (vi) Science Direct; (vii) China National Knowledge Infrastructure (CNKI); and (viii) VIP Information, from inception to 31 July

2013. No language or publication type restrictions were applied. The following keywords and combinations thereof were used: “reinfection”, “soil-transmitted helminths”, “multiparasitism”, “polyparasitism”, “infection intensity”, “hookworm”, “*Ascaris*”, “*Trichuris*”, “nutrition”, “undernutrition”, “malnutrition”, “iron”, “zinc”, “vitamin”, “nutrition supplementation” and “micronutrient supplementation”. The detailed search strategies are described in Supplementary table 12.1. Bibliographies of all identified studies were checked manually for potential additional references.

12.3.3. Data collection and analysis

Selection of studies

The study selection process was performed by the first author. Potentially relevant studies were identified by screening the titles and, if available, the abstracts of publications. The full texts were assessed if the relevance of a publication could not be judged from its title or abstract. Finally, the full texts of all potentially relevant studies were evaluated against the predefined inclusion criteria.

Data extraction

The full text of all selected studies were reviewed and information on the study period, location, design, objectives, method of recruitment, sample size, socio-demographic characteristics of, and inclusion criteria for, study participants were extracted. In addition, information on the soil-transmitted helminth species and nutritional supplementation used (type, duration, dosage, frequency, type of control group and presence of co-interventions, if any) were gathered, along with both primary and secondary outcomes.

Data analysis

Where relevant data were available, unadjusted odds ratios (ORs) with 95% confidence intervals (CIs), comparing prevalence rates among individuals who received nutritional supplementation/were well-nourished, and those who received placebo/were under-nourished, were calculated. Fixed-effects meta-analyses were conducted for *A. lumbricoides*, *T. trichiura*, hookworm and, when the results were not species-specific, soil-transmitted helminths combined. Unweighted ORs were pooled according to the type of nutritional supplementation/natural nutrition status of the host and an overall OR was also generated. An OR of less than 1.0 indicates a decrease in the odds of being (re-)infected with soil-transmitted helminths among individuals who received nutritional

supplementation/were well-nourished. All statistical analyses were performed with STATA version 10.0 (STATA Corp.; College Station, TX, USA).

In addition, qualitative content analysis was conducted for all studies included in the systematic review. Individual outcome measures reported in the articles were summarised and categorised into “strong positive effect”, “moderate-to-weak positive effect”, and “negative effect”. Outcome measures with a P -value <0.05 were considered to be of statistical significance. The categories were defined as: (i) “strong positive effect” if the intervention/well-nourished group showed a more than 2-fold and statistically significant improvement compared to the control/undernourished group; (ii) “moderate-to-weak positive effect” if the improvements were between nil and 2-fold or more than 2-fold but not statistically significant; and (iii) “negative effect” whenever outcome measures worsened in the intervention/well-nourished group as compared to the control/undernourished group. Consequently, an article could have outcome measures listed in different categories if it contained results of different effect size, e.g. for different soil-transmitted helminth species or different interventions.

Quality assessment of included studies

Following the GRADE rating system (Balshem *et al.* 2011), outcomes of each study were checked for consistency, precision, directness and the magnitude of the observed effect. The risk of bias was assessed by examining the method of allocation to different study groups; whether randomization, concealment (protection of the randomization process to ensure treatment allocation is not known prior to study initiation) or blinding of participants, providers and outcome assessors to the type of intervention received, were performed; the presence of selection, measurement and reporting bias; and loss to follow-up. The evidence quality of the identified studies was rated as high, moderate, low or very low, according to the GRADE guidelines. The full review protocol is available from the corresponding author.

12.4. Results

12.4.1. Characteristics of studies

The literature searches yielded a total of 13,893 hits (Figure 12.2). Based on the titles or abstracts, only 20 studies were identified for inclusion. The full texts of all identified studies were retrieved and assessed. Screening of the bibliographies of these

studies revealed an additional 3 studies. Among these 23 studies, 8 were excluded (cross-sectional survey, n=1; reviews, n=2; interactions between malnutrition and immunity with no specific reference to soil-transmitted helminths, n=2; small case series of 3-12 individuals, n=2; and unable to obtain the full text, n=1) (Supplementary table 12.2).

Among the 15 studies included in our final analysis, 6 had been conducted in Central America (Grazioso *et al.* 1993; Saldiva *et al.* 2002; Long *et al.* 2006, 2007; Payne *et al.* 2007; Halpenny *et al.* 2013); 4 in Africa (Kightlinger *et al.* 1996; Olsen *et al.* 2000; Olsen *et al.* 2003; Nchito *et al.* 2009); 3 in Asia (Hesham Al-Mekhlafi *et al.* 2008; Nga *et al.* 2009, 2011); and 2 in South America (Hagel *et al.* 1995, 1999). These studies were all published in English.

Characteristics of the 15 studies are listed in the summary of findings (SoF) table (Table 12.1 and Supplementary table 12.3). Eight studies were randomised controlled trials; 2 of the publications (Nga *et al.* 2009, 2011) actually contained data from the same randomised controlled trial, with different components of the findings published in each of them. In addition, there were 7 prospective cohort studies. Data pertaining to *A. lumbricoides* were available from each study, while 7 studies additionally reported data on *T. trichiura* and 6 on hookworm. Fourteen studies included children only, aged between 5 months and 15 years. One study (Olsen *et al.* 2000) focused on adolescents and adults (age range: 16-63 years). The design of 7 studies included the administration of albendazole or mebendazole to participants at baseline and monitoring subjects for re-infection with soil-transmitted helminths over a period of 4 to 15 months, during which regular nutritional supplementation or placebo was provided. Nutritional supplementation included multi-micronutrient biscuits or tablets, vitamin A given as retinol solution, zinc administered as zinc methionine solution or iron taken as ferrous dextran tablets. In 2 studies (Long *et al.* 2006, 2007), young children, aged 5-15 months, were given only nutritional supplementation or placebo, without preventive chemotherapy, and followed for 12-15 months for *A. lumbricoides* infection or immune responses against this nematode. Finally, six studies evaluated albendazole, mebendazole or oxantel/pyrantel treatments with evaluation of the participants, stratified by their natural nutrition states, occurring 8 months (Hagel *et al.* 1999) or 12 months (Hagel *et al.* 1995) after the treatment period, while in another study, albendazole was administered to children who were followed, in their natural nutrition states, over 2 treatment and re-infection cycles (Halpenny *et al.* 2013).

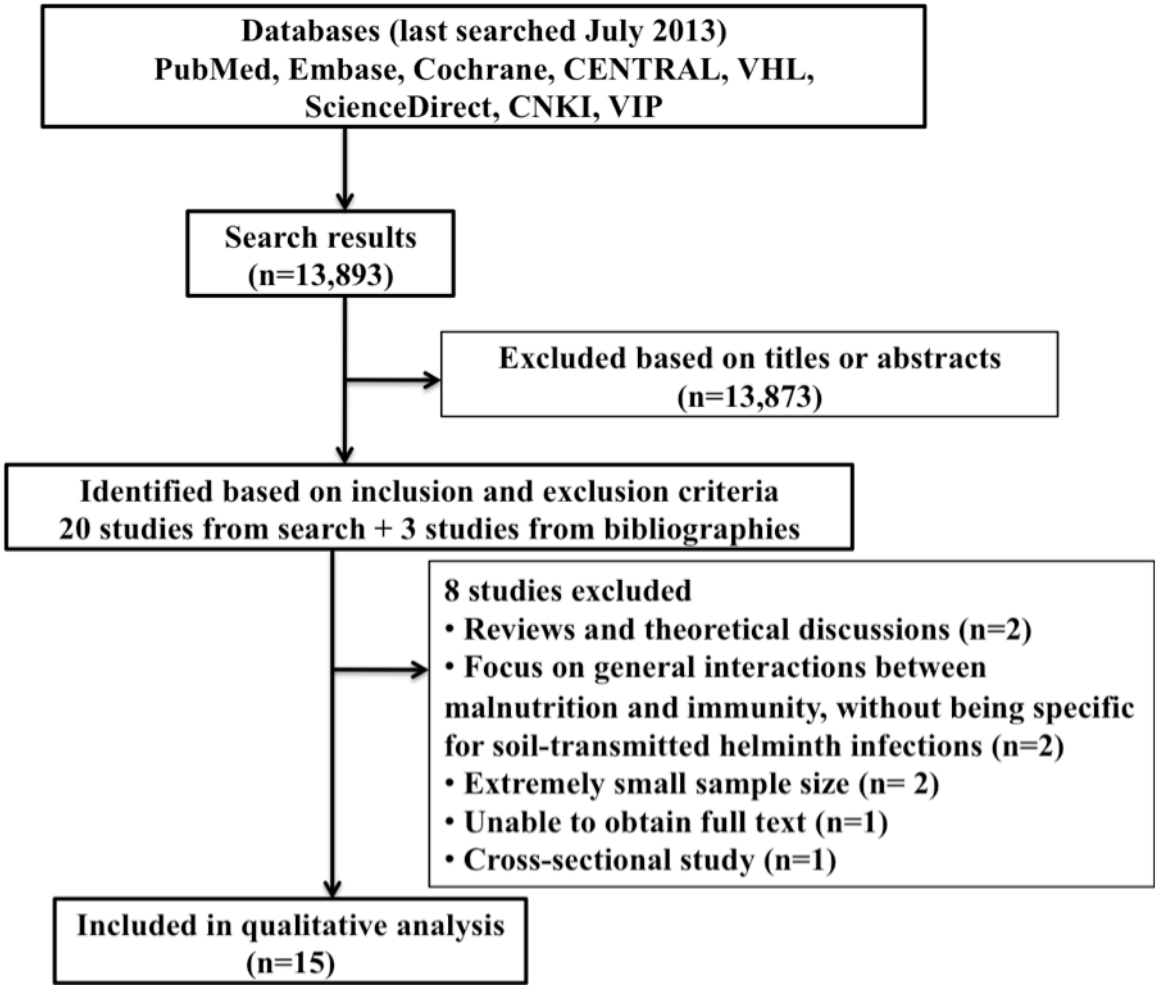


Figure 12.2. Search strategy for the identification of studies examining the influence of nutrition on re-infection with soil-transmitted helminths in humans.

12.4.2. Quality of evidence in included studies

Among the 15 studies included in this review, only 4 had evidence graded as high quality (no serious limitation, inconsistency, indirectness and imprecision were present and no risk of bias was detected). No study had evidence of moderate quality. Evidence in six studies was given a low quality rating and evidence from another 5 were determined to be of very low quality (Table 12.2). When stratified according to the impact of the outcome measures, the 8 studies with strong positive impact contained 3 (27%) high, 4 (45%) low and 1 (27%) very low quality evidence; the 9 studies with moderate-to-weak positive effect were made up of 2 (22%) high, 4 (44%) low and 3 (33%) very low quality evidence; and the 6 studies with negative effect included 1 (17%) high, 4 (67%) low and 1 (17%) very low quality evidence.

12.4.3. Influence of nutrition on soil-transmitted helminth infections

Multi-micronutrients

Multi-micronutrients, taken in the form of biscuits or tablets, lowered the re-infection rate with *A. lumbricoides* (OR: 0.76, 95% CI: 0.59-0.98, Figure 12.3A) and *T. trichiura* (OR: 0.76, 95% CI: 0.55-1.06, Figure 12.3B) but failed to reach statistical significance (Olsen *et al.* 2003; Nga *et al.* 2009; Nchito *et al.* 2009); the quality of this evidence is moderate. Slightly higher re-infection rates were observed for hookworm in subjects given micronutrients, again without statistical significance (OR: 1.08, 95% CI: 0.61-1.92, Figure 12.3C). The quality of this evidence is moderate. Regarding re-infection intensity, 2 studies containing moderate quality evidence reporting moderate-to-weak positive effects were identified for all three intestinal helminth species (1,443 participants) (Olsen *et al.* 2003; Nga *et al.* 2011), and 1 study of low quality evidence reporting negative effects was identified for *A. lumbricoides* (215 participants) (Nchito *et al.* 2009). In terms of secondary outcome measures, multi-micronutrients had a strong positive effect on reducing the odds of anaemia, zinc and iodine deficiencies, but only a moderate-to-weak positive effect on growth and cognition in 1 study (466 participants, high quality evidence) (Nga *et al.* 2009, 2011).

Iron

Iron tablets probably decrease the re-infection rate with *A. lumbricoides* (OR: 0.75, 95% CI: 0.54-1.05, Figure 12.3A), but the quality of this evidence is low (Olsen *et*

al. 2000; Nchito *et al.* 2009). Iron might also reduce the infection intensity of *A. lumbricoides* (2 studies of moderate-to-weak positive effects, 544 participants, low quality evidence) (Olsen *et al.* 2000; Nchito *et al.* 2009). It is not clear what effect iron has on the re-infection rate and infection intensity with *T. trichiura* and hookworm, as only 1 study with moderate-to-weak and negative effects (329 participants, low quality evidence) was identified (Olsen *et al.* 2000).

Vitamin A

Vitamin A supplementation showed no effect on re-infection with *A. lumbricoides* (OR: 1.11, 95% CI: 0.79-1.56, Figure 12.3A; quality of evidence is moderate) (Payne *et al.* 2007; Long *et al.* 2007). With regards to a reduction of *A. lumbricoides* infection intensity, only 1 low-quality evidence with moderate-to-weak positive effects was found (328 participants) (Payne *et al.* 2007). We identified no studies examining the impact of vitamin A on *T. trichiura* and hookworm re-infection. In terms of secondary outcome measures, vitamin A increased the odds of having improved levels of IL-4, which is part of the Th2 response against helminths, in 1 study (strong positive effect, 127 participants, high quality evidence) (Long *et al.* 2006).

Zinc

Zinc might have a negative effect on the rate of re-infection with *A. lumbricoides* as 1 study (707 participants, high quality evidence) (Long *et al.* 2007) showed an increase in the prevalence of this parasite in children who took zinc tablets and another study (130 participants, very low quality evidence) (Grazioso *et al.* 1993) also highlighted that zinc-supplemented children fared worse than their placebo counterparts in terms of soil-transmitted helminth re-infection (*A. lumbricoides* and *T. trichiura* were treated collectively and no stratification of results is available in the published article). No other studies investigating the impact of zinc on *T. trichiura* and hookworm re-infection were identified.

Nutritional combinations

Nutritional combination therapies might slightly increase the re-infection rate of *A. lumbricoides*. We identified 2 studies, one combining iron and multi-micronutrients (OR: 1.26, 95% CI: 0.89-1.78, Figure 12.3A) (Nchito *et al.* 2009), and the second, vitamin A plus zinc (OR: 1.29, 95% CI: 0.80-2.06, Figure 12.3A) (Long *et al.* 2007). In terms of secondary outcomes, a combination of zinc and vitamin A reduced the mean duration of *A. lumbricoides* infection (moderate-to-weak positive effect) and lowered the

incidence of *A. lumbricoides*-associated diarrhoea (strong positive effect) in another study (707 participants, high quality evidence) (Long *et al.* 2007).

Natural nutrition status of the host

The natural nutrition status of the host might have an impact on soil-transmitted helminth re-infection according to 1 low quality evidence which showed strong positive effects (279 participants) (Saldiva *et al.* 2002; Hesham Al-Mekhlafi *et al.* 2008; Halpenny *et al.* 2013), and 4 very low quality evidence (1 with strong positive and 3 with moderate-to-weak positive effects, 1,409 participants) (Kightlinger *et al.* 1996; Hagel *et al.* 1999). Indeed, these 5 studies found that children considered to be well nourished had lower re-infection rates and intensities of soil-transmitted helminths compared to their under-nourished peers. In terms of secondary outcome measures, hosts with a better nutrition status had higher levels of anti-*Ascaris* IgE levels (strong positive effect) than those with poor nutrition indicators in 1 study (85 participants, low quality evidence) (Hagel *et al.* 1995).

Table 12.1. Summary of findings (SoF) for 15 studies meeting the inclusion criteria of the systematic review of studies on undernutrition and re-infection with soil-transmitted helminths.

Characteristics of studies			
Study Design ^a ; location; sample size; intestinal helminth examined ^b	Study subjects	Intervention/control arms	Outcomes
1) Nga <i>et al.</i> 2011; factorial RCT; Vietnam; N=466; A, T, H	6- to 8-year- old children	i) Multi-micronutrient biscuits fortified with iron, zinc, iodine and vitamin A given on 5 days per week for 4 months and albendazole (400 mg, single dose) at baseline ii) Multi-micronutrient biscuits and placebo, identical looking to albendazole iii) Non-fortified, identical looking biscuits and albendazole at baseline iv) Non-fortified, identical looking biscuits and albendazole placebo	Primary: i) Moderate-to-weak positive effect in the reduction of infection intensity of all intestinal helminth species in children taking ‘fortified biscuits and albendazole’ <i>versus</i> ‘albendazole alone’ Secondary: Moderate-to-weak positive effect in improving growth and cognition in children receiving fortified biscuits
2) Nga <i>et al.</i> 2009; factorial RCT; Vietnam; N=466; A, T, H	6- to 8-year- old children	ii) Multi-micronutrient biscuits fortified with iron, zinc, iodine and vitamin A given on 5 days per week for 4 months and albendazole (400 mg, single dose) at baseline ii) Multi-micronutrient biscuits and placebo, identical looking to albendazole iii) Non-fortified, identical looking biscuits and albendazole at baseline iv) Non-fortified, identical looking biscuits and	Primary: i) Strong positive effect in the reduction of prevalence of all intestinal helminth species in children taking ‘fortified biscuits and albendazole’ <i>versus</i> ‘albendazole alone’ Secondary: Strong positive effect in reducing anaemia, zinc and iodine deficiencies in children receiving fortified biscuits

albendazole placebo			
3) Nchito <i>et al.</i> 2009; factorial RCT; Zambia; N=215; A	7- to 15- year-old children	<p>i) Albendazole (400 mg) given to all study participants on 2 consecutive days at baseline</p> <p>ii) Multi-micronutrient tablet fortified with vitamin A, B₁, B₂, B₆, B₁₂, C, D and E, niacin, folic acid, zinc, iodine, copper and selenium every school day for 10 months and ferrous dextran tablet (equivalent to 60 mg of elemental iron) every school day for 10 months</p> <p>iii) Multi-micronutrient tablet and placebo iron tablet</p> <p>iv) Placebo multi-micronutrient tablet and ferrous dextran tablet</p> <p>v) Placebo multi-micronutrient tablet and placebo iron tablet</p>	<p>Primary: i) Moderate-to-weak effects in the reduction of prevalence of <i>A. lumbricoides</i> in children taking ‘iron only’ and ‘multi-micronutrients only’ <i>versus</i> ‘placebo’. Negative effect in the reduction of prevalence of <i>A. lumbricoides</i> in children taking ‘iron with multi-micronutrients’ <i>versus</i> ‘placebo’</p> <p>ii) Moderate-to-weak positive effect in the reduction of infection intensity of <i>A. lumbricoides</i> in children taking ‘iron only’ <i>versus</i> ‘placebo’. Negative effects in the reduction of infection intensity of <i>A. lumbricoides</i> in children taking ‘iron with multi-micronutrients’ and ‘multi-micronutrients only’ <i>versus</i> ‘placebo’</p>
4) Long <i>et al.</i> 2007; factorial RCT; Mexico; N=707; A	6- to 15- month-old children	<p>i) Vitamin A (given as 20,000 IU of retinol for children <1 year and 45,000 IU for children >1 year) every 2 months for 1 year and zinc methionine (equivalent to 20 mg of elemental zinc)</p> <p>ii) Zinc methionine only</p> <p>iii) Vitamin A only</p> <p>iv) Placebo</p>	<p>Primary: i) Strong positive effect in the reduction of prevalence of <i>A. lumbricoides</i> in children taking ‘zinc alone’ <i>versus</i> ‘placebo’. Negative effects in the reduction of prevalence of <i>A. lumbricoides</i> in children taking ‘vitamin A with zinc’ and ‘vitamin A alone’ <i>versus</i> placebo</p> <p>Secondary: i) A combination of vitamin A and zinc had a moderate-to-weak positive effect on the reduction of <i>A. lumbricoides</i> infection duration and a strong positive effect on reduction of <i>A. lumbricoides</i>-associated diarrhoea</p>
5) Long <i>et al.</i> 2006; RCT; Mexico; N=127;	5- to 15- month-old children	<p>i) Vitamin A (given as 20,000 IU of retinol for children <1 year and 45,000 IU for children >1 year) every 2 months for 15 months</p> <p>ii) Placebo</p>	<p>Secondary: i) Strong positive effect in the increase of interleukin 4 (IL-4) levels in vitamin A supplemented children <i>versus</i> placebo</p>

A			
6) Olsen <i>et al.</i> 2003; RCT; Kenya; N=977; A, T, H	8- to 18- year-old children	<p>i) Albendazole (600 mg, single dose) given to all children at baseline and 4 weeks after baseline (600 mg, single dose) if child was still infected</p> <p>ii) Multi-micronutrient tablet fortified with vitamin A, B₁, B₂, B₆, B₁₂, C, D and E, niacin, folic acid, zinc, iodine, copper, iron and selenium every school day for 11 months</p> <p>iii) Placebo, identical looking to the multi-micronutrient tablet</p>	<p>Primary: i) For children taking ‘multi-micronutrients’ <i>versus</i> ‘placebo’, moderate-to-weak positive effects in the reduction of prevalence of <i>A. lumbricoides</i> and hookworm and negative effect in the reduction of prevalence of <i>T. trichiura</i></p> <p>ii) For children taking ‘multi-micronutrients’ <i>versus</i> ‘placebo’, moderate-to-weak positive effects in the reduction of infection intensity of all intestinal helminth species</p>
7) Olsen <i>et al.</i> 2000; RCT; Kenya; N=329; A, T, H	4- to 15- year-old children (n=200) and 16- to 63- year-old adolescents and adults (n=129)	<p>i) Albendazole (400 mg, once a day for 3 consecutive days) at baseline for all individuals and if any individual was still infected between 3 and 6 months after baseline, re-treatment (400 mg, single dose) was given</p> <p>ii) Ferrous dextran tablet (equivalent to 60 mg of elemental iron) twice weekly for 12 months</p> <p>iii) Placebo identical looking to the ferrous dextran tablet</p>	<p>Primary: i) For children taking ‘iron’ <i>versus</i> ‘placebo’, moderate-to-weak positive effects in the reduction of prevalence of all intestinal helminth species and in the reduction of infection intensity of hookworm. Negative effects in the reduction of infection intensity of <i>A. lumbricoides</i> and <i>T. trichiura</i></p> <p>ii) For adolescents/adults taking ‘iron’ <i>versus</i> ‘placebo’, strong positive effects in the reduction of prevalence of <i>A. lumbricoides</i> and <i>T. trichiura</i> and moderate-to-weak positive effect in the reduction of prevalence of hookworm. In terms of infection intensity reduction, negative effects for <i>A. lumbricoides</i> and <i>T. trichiura</i> and moderate-to-weak positive effect for hookworm</p>
8) Grazioso <i>et al.</i> 1993; RCT; Guatemala; N=130; A, T	65- to 95- month-old children	<p>i) Mebendazole (100 mg twice daily for 3 days) at baseline for all individuals</p> <p>ii) Tablet containing zinc (10 mg) and other micronutrients, namely vitamin A, E, C, B₆, B₁₂ and D, folic acid, thiamin, riboflavin, niacinamide, pantothenic acid, iron, copper, iodine, selenium, chromium and magnesium, given on every school</p>	<p>Primary: i) Negative effect in the reduction of prevalence of <i>A. lumbricoides</i> and <i>T. trichiura</i> (mentioned collectively) in children taking ‘zinc’ <i>versus</i> ‘placebo’</p>

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		day for 120-150 days	
		iii) Different colour-coded tablets, containing all the micronutrients, except for zinc, found in the intervention tablet	
9) Halpenny <i>et al.</i> 2013; CP; Panama; N=87-279; A, H	<5-year-old children	i) Cycle 1: Albendazole (200-400 mg depending on age, single dose) to all children >12 months at baseline. Children followed up for 9 months ii) Cycle 2: Albendazole (200-400 mg depending on age, single dose) to all children >12 months at baseline. Children who remained infected with at least 1 soil-transmitted helminth were given another single dose of albendazole. Children followed up for 6 months	Primary: i) Strong positive effect in reduction of <i>A. lumbricoides</i> infection intensity at the end of cycle 1 in children with higher height-for-age (HAZ) <i>versus</i> their peers with lower HAZ score ii) Strong positive effect in reduction of hookworm infection intensity at the end of cycle 2 in children with higher height-for-age (HAZ) <i>versus</i> their peers with lower HAZ score
10) Hesham Al-Mekhlafi <i>et al.</i> 2008; CP; Malaysia; N=120; A, T, H	7- to 12-year-old children	i) Albendazole (400 mg, once a day for 3 consecutive days) for all children at baseline. Children followed for 6 months to investigate predictors of re-infection	Primary: i) Moderate-to-positive effects in the reduction of prevalence of <i>A. lumbricoides</i> , <i>T. trichiura</i> and hookworm (mentioned collectively) in non-stunted children <i>versus</i> stunted children
11) Payne <i>et al.</i> 2007; CP; Panama; N=328; A	12- to 60-month-old children	i) One time supplementation with vitamin A (60 mg retinol) given by the Ministry of Health ii) Albendazole (400 mg, single dose) for all children at baseline. Children were followed at 3 and 5 months post-treatment	Primary: i) Moderate-to-weak positive effect in the reduction of <i>A. lumbricoides</i> prevalence and infection intensity in vitamin A supplemented children <i>versus</i> non-supplemented ones
12) Saldiva <i>et al.</i> 2002; CP;	1- to 10-year-old	i) Mebendazole (triple doses at baseline and repeated 15 days after). Children were followed at 1	Primary: i) Moderate-to-weak positive effect in the reduction of prevalence of <i>A. lumbricoides</i> and <i>T. trichiura</i> (mentioned collectively) in eutrophic children <i>versus</i>

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Brazil; N=585; A, T	children	year post-treatment	undernourished children
13) Hagel <i>et al.</i> 1999; CP; Venezuela; N=85; A	6- to 11- year-old children	i) Oxantel/pyrantel (20 mg/kg) monthly for 12 months for all children. Children were followed at 8 months after the 12 months of treatment	Primary: i) Strong positive effect in the reduction of prevalence of <i>A. lumbricoides</i> in children >10 th percentile for height <i>versus</i> children ≤10 th percentile for height
14) Kightlinger <i>et al.</i> 1996; CP; Madagascar; N=360-619; A	4- to 10- year-old children	i) Mebendazole (500 mg, single dose) was given to all children at baseline. Children were followed at the end of 12 months, when they were given pyrantel pamoate (11 mg/kg) and 48-hour worm expulsions were performed	Primary: i) Moderate-to-weak positive effect in the reduction of infection intensity of <i>A. lumbricoides</i> in the best-nourished children <i>versus</i> children with reduced growth indicators
15) Hagel <i>et al.</i> 1995; CP; Venezuela; N=85; A	6- to 11- year-old children	i) Oxantel/pyrantel (20 mg/kg) monthly for 12 months for all children. Children were followed at the end of the 12 months of treatment	Secondary: i) Strong positive effect in an increase of anti- <i>Ascaris</i> IgE levels in well-nourished children <i>versus</i> under-nourished children

^a CP, cohort prospective; RCT, randomised controlled trial

^b A, *A. lumbricoides*; H, hookworm ; T, *T. trichiura*

Table 12.2. GRADE evidence profile (EP) for the 15 studies included in the systematic review.

Study	Quality assessment of evidence					Quality grading
	Limitation	Inconsistency	Indirectness	Imprecision	Risk of bias	
1) Nga <i>et al.</i> 2011	No serious limitation	No serious inconsistency	No serious indirectness	No serious imprecision	None detected	⊕ ⊕ ⊕ ⊕ High
2) Nga <i>et al.</i> 2009	No serious limitation	No serious inconsistency	No serious indirectness	No serious imprecision	None detected	⊕ ⊕ ⊕ ⊕ High
3) Nchito <i>et al.</i> 2009	Serious limitation (sample size used for analysis was smaller than that required for statistical significance; mean number of supplementation tablets taken was only 50% of tablets provided)	Serious inconsistency (administration of albendazole at baseline was not stated under study design but was mentioned under results)	No serious indirectness	No serious imprecision	Serious risk of bias (47% of children were lost to follow-up; method of recruitment and inclusion/exclusion criteria were not mentioned)	⊕ ⊕ ○ ○ Low
4) Long <i>et al.</i> 2007	No serious limitation	No serious inconsistency	No serious indirectness	No serious imprecision	None detected	⊕ ⊕ ⊕ ⊕ High
5) Long <i>et al.</i> 2006	No serious limitation	No serious inconsistency	No serious indirectness	No serious imprecision	None detected	⊕ ⊕ ⊕ ⊕ High
6) Olsen <i>et al.</i> 2003	Serious limitation (compliance rates	Serious inconsistency (the	No serious	No serious	Serious risk of bias (number of stool	⊕ ⊕ ○ ○

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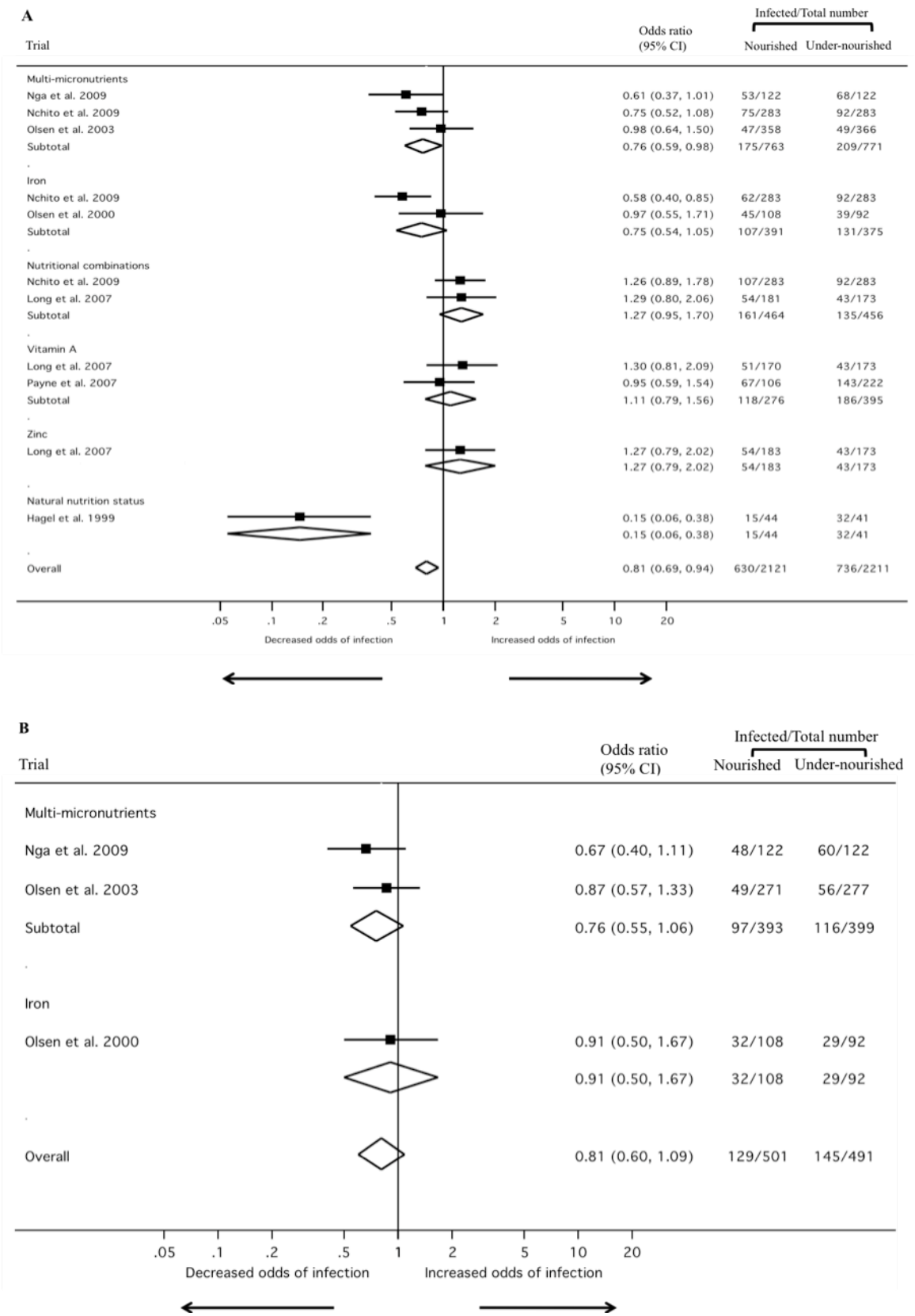
	for the multi-micronutrient tablet and placebo were low at 46%)	allocation of anthelmintic treatment and placebo was not clear)	indirectness	imprecision	samples collected for each child varied)	Low
7) Olsen <i>et al.</i> 2000	No serious limitation	Serious inconsistency (reporting of results was not consistent for all the helminth species)	No serious indirectness	No serious imprecision	Serious risk of bias (method of recruitment and blinding procedures not mentioned; number of stool samples collected varied at each follow-up)	⊕ ⊕ ○ ○ Low
8) Grazioso <i>et al.</i> 1993	No serious limitation	Serious inconsistency (data reported under abstract is different from that found in the results section)	No serious indirectness	Serious imprecision (stratification of results according to soil-transmitted helminth species was not performed)	Very serious risk of bias (number of intervention days not clear; reporting of primary outcome measures were not complete)	⊕ ○ ○ ○ Very low
9) Halpenny <i>et al.</i> 2013	Serious limitation (compliance rate for albendazole at both treatment cycles was low at 48%)	No serious inconsistency	No serious indirectness	No serious imprecision	None detected	⊕ ⊕ ○ ○ Low
10) Hesham Al-Mekhlafi <i>et al.</i> 2008	No serious limitation	Serious inconsistency (reporting of sample size was not consistent throughout the	No serious indirectness	Serious imprecision (stratification of results according to soil-transmitted helminth species	None detected	⊕ ○ ○ ○ Very low

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		study)		was not performed)		
11) Payne <i>et al.</i> 2007	No serious limitation	No serious inconsistency	No serious indirectness	No serious imprecision	Serious risk of bias (vitamin A supplemented children came from families with significantly higher income and latrine access than the non-supplemented children; 34% children were lost to follow-up)	⊕ ⊕ ○ ○ Low
12) Saldiva <i>et al.</i> 2002	No serious limitation	Serious inconsistency (stratification of undernourished and eutrophic children not clear)	No serious indirectness	Serious imprecision (stratification of results according to soil-transmitted helminth species was not performed)	None detected	⊕ ○ ○ ○ Very low
13) Hagel <i>et al.</i> 1999	No serious limitation	No serious inconsistency	No serious indirectness	No serious imprecision	Serious risk of bias (poverty level as a confounding factor was not taken into account during data analysis)	⊕ ○ ○ ○ Very low
14) Kightlinger <i>et al.</i> 1996	No serious limitation	Serious inconsistency (number of children included for analysis varied for different outcome)	No serious indirectness	No serious imprecision	Serious risk of bias (about 41% children were lost to follow-up)	⊕ ○ ○ ○ Very low

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measures)						
15) Hagel <i>et al.</i> 1995	No serious limitation	No serious inconsistency	No serious indirectness	No serious imprecision	None detected	⊕ ⊕ ○ ○ Low



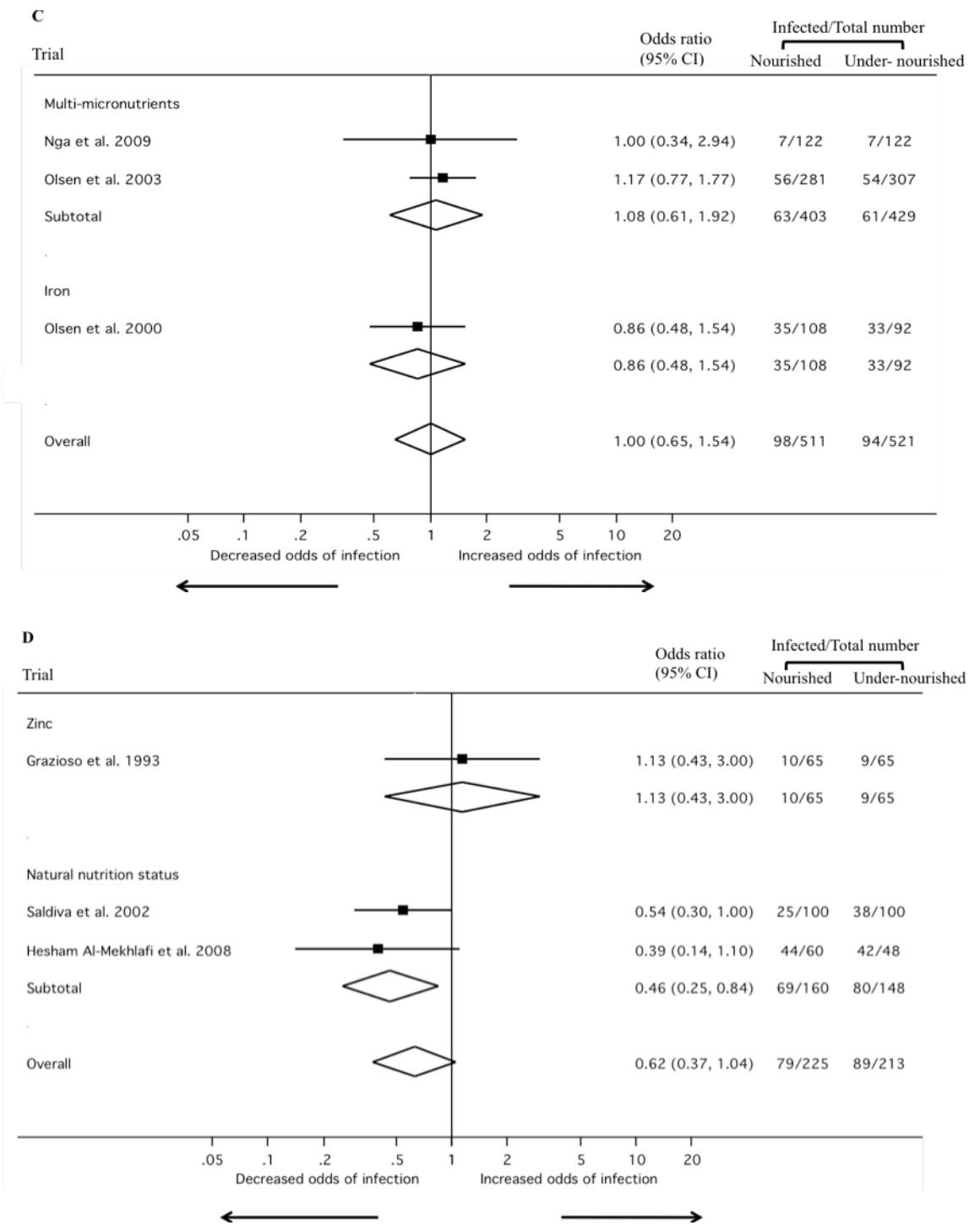


Figure 12.3. Meta-analysis examining the association of nutritional supplementation/host’s natural nutrition status with *A. lumbricoides* (A), *T. trichiura* (B), hookworm (C) and soil-transmitted helminths combined (D).

12.5. Discussion

There is a considerable body of literature on the effects of macro- and micronutrients on host immune function and their association with infectious diseases (Tomkins *et al.* 1989; Scrimshaw *et al.* 1997; Carr *et al.* 1999; Koski *et al.* 2001; Katona *et al.* 2008; Maggini *et al.* 2010). However, little is known whether nutritional deficiencies have an effect on the susceptibility to infection and re-infection with soil-transmitted helminths. To fill this gap, we conducted a systematic review examining the influence of nutrition on infection and re-infection with soil-transmitted helminths. We focused particularly on the use of nutritional supplementation to prevent re-infection following anthelmintic treatment. The results from our systematic review indicate that first, only few studies are available and, second, most of the evidence on the effects of nutritional supplementation and undernutrition on re-infection with soil-transmitted helminths is of low quality. Among the various nutritional supplementation interventions reviewed, multi-micronutrients seemed to have the clearest effect in terms of lowering re-infection rates and intensity of soil-transmitted helminths, whereas consumption of zinc or vitamin A alone might have a negative effect on these two outcome measures. With regard to the natural nutrition status of the host, the general trend observed was that individuals with poor nutrition indicators experienced higher re-infection rates and intensities than their well-nourished counterparts. However, the risk of confounding is higher in studies focusing on the natural nutrition status rather than controlled supplementation following randomization. Hence, findings from the former studies have to be interpreted with caution.

The evidence reported here must be seen in conjunction with the strengths and limitations of our systematic review. In terms of strength, we conducted a broad search including 8 major electronic scientific literature databases that were systematically reviewed for relevant articles, complemented with hand-searches of bibliographies of identified articles. The reporting of the review was done based on the PRISMA guidelines (Moher *et al.* 2009), while the GRADE system (Balshem *et al.* 2011) was adopted for grading the quality of the reported evidence. A combination of meta-analysis and qualitative content analysis was adopted to ensure a comprehensive review. Due to the small number of studies identified, most of which with low quality evidence that is statistically insignificant, the unweighted pooled ORs should be interpreted with caution. Therefore, it is also statistically irrelevant to detect heterogeneity with Moran's I^2 or

publication bias with the Egger's test. However, a potential publication bias was noted based on the forest plots, where studies with smaller sample sizes presented more significant results than studies with larger sample sizes. Finally, no "grey literature" and experts' opinions were included as the quality and strength of evidence of these sources is usually lower than that of articles published in the peer-reviewed literature.

There are several shortcomings in the included studies. None of the studies investigated the effect of nutritious whole foods as an intervention. Nutrients delivered by whole foods derived from the biological environment of the study population might have a distinct impact compared to synthetic supplements, since consumption of a broad range of nutrients from their natural sources might aid in their absorption and assimilation into the body (Jacobs *et al.* 2007). Earlier work (Ahmann *et al.* 1933; Tripathy *et al.* 1971) did attempt to improve on whole diets and assess their impact on hookworm infection but the ill-controlled dietary changes render it difficult to appreciate these results. Second, the use of multi-micronutrient-fortified biscuits by Nga and co-workers (Nga *et al.* 2009, 2011) is a compromise between the two ends of the spectrum, allowing artificial nutrients to be delivered through food. Unfortunately, this is the only group that employed this strategy and more evidence is needed to confirm the efficacy of such a strategy in preventing re-infection with soil-transmitted helminths.

It must be noted that nutrients often have interactions that affect their absorption and presumably their impact on soil-transmitted helminth re-infection. Antagonistic interactions were observed in two studies pertaining to *A. lumbricoides* infection (Long *et al.* 2007; Nchito *et al.* 2009). However, in one of these studies (Long *et al.* 2007), vitamin A and zinc were also shown to work synergistically and better than placebo or taking the supplements individually in reducing the mean duration of *A. lumbricoides* infection and the incidence of diarrhea. In order to fully exploit the potential of nutritional supplementation for reducing re-infection with soil-transmitted helminths, the careful identification of synergistic combinations of supplements is thus required.

On a more fundamental note, it is currently unclear how long it will take for a reliable source of nutrition to become utilised for the building up of immune defenses and not for catching up on retarded growth. Many of the populations from the studies reviewed here have suffered from chronic undernutrition and high prevalence of soil-transmitted helminths and other infectious diseases, and hence, the treatment, nutritional supplementation given and the observation period might not be adequate or sufficient for

the body to recover from the accumulated growth retardation, to wipe out infections and to strengthen the immune system at the same time. Therefore, more rigorous chemotherapies, such as triple-dose regimens or combination therapies (Olsen 2007; Keiser *et al.* 2010; Knopp *et al.* 2010; Steinmann *et al.* 2011; Yap *et al.* 2013) repeated over an extended period of time as well as continuous nutritional supplementation or markedly improved food supplies both in terms of quality and quantity might be needed to allow study subjects to grow and build up their immune defense. Such a situation might be more suitable for an accurate test of the impact of nutritional supplementation on re-infection with soil-transmitted helminths.

12.6. Conclusion

We conclude that the current evidence-base for the effect of nutrition on re-infection with soil-transmitted helminths is weak and of low quality. Hence, no guidelines on nutrition management with or without preventive chemotherapy can be derived. In order to generate the required evidence for policy makers to base their recommendations on, future studies should focus on having a rigorous study design, and consider whole foods, the entire diet as well as combination supplementation as intervention tools. Making sure that the body has sufficient time to recover from undernutrition and soil-transmitted helminth infection before the final evaluation of the intervention is another requirement. Finally, it is important to realise that multi-pronged approaches are probably necessary to prevent and control the negative effects of soil-transmitted helminth infections, including anthelmintic drugs, safe water and sanitation, proper hygiene habits and, possibly, improved nutrition.

12.7. Acknowledgments

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12.8. References

- Ahmann CF & Bristol LM (1933) The effect of diet on the worm burden of children infected with *Necator americanus* and *Ascaris lumbricoides*. *South Med J.* **16**, 959-62.
- Balshem H, Helfand M, Schunemann HJ *et al.* (2011) GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol.* **64**, 401-6.
- Bethony J, Brooker S, Albonico M *et al.* (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet.* **367**, 1521-32.
- Brooker S (2010) Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers - a review. *Int J Parasitol.* **40**, 1137-44.
- Brooker S, Clements ACA & Bundy DAP (2006) Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol.* **62**, 221-61.
- Carr AC & Frei B (1999) Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am J Clin Nutr.* **69**, 1086-107.
- Chammartin F, Scholte RGC, Guimarães LH, Tanner M, Utzinger J & Vounatsou P (2013) Soil-transmitted helminth infection in South America: a systematic review and geostatistical meta-analysis. *Lancet Infect Dis.* **13**, 507-18.
- Cox FE (2002) History of human parasitology. *Clin Microbiol Rev.* **15**, 595-612.
- Crompton DWT & Nesheim MC (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr.* **22**, 35-59.
- FAO (2012) Undernourishment around the world in 2012. Rome, Food and Agriculture Organization of the United Nations.
- Gershwin ME, German JB & Keen CL (2000) *Nutrition and immunology: principles and practice*, Humana Press.
- Grazioso CF, Isalgue M, de Ramirez I, Ruz M & Solomons NW (1993) The effect of zinc supplementation on parasitic reinfestation of Guatemalan schoolchildren. *Am J Clin Nutr.* **57**, 673-8.
- Hagel I, Lynch NR, Di Prisco MC *et al.* (1999) Helminthic infection and anthropometric indicators in children from a tropical slum: *Ascaris* reinfection after anthelmintic treatment. *J Trop Pediatr.* **45**, 215-20.
- Hagel I, Lynch NR, Di Prisco MC, Sanchez J & Perez M (1995) Nutritional status and the IgE response against *Ascaris lumbricoides* in children from a tropical slum. *Trans R Soc Trop Med Hyg.* **89**, 562-5.
- Hall A (2007) Micronutrient supplements for children after deworming. *Lancet Infect Dis.* **7**, 297-302.

- Hall A, Zhang Y, Macarthur C & Baker S (2012) The role of nutrition in integrated programs to control neglected tropical diseases. *BMC Med.* **10**, 41.
- Halpenny CM, Paller C, Koski KG, Valdés VE & Scott ME (2013) Regional, household and individual factors that influence soil transmitted helminth reinfection dynamics in preschool children from rural indigenous Panamá. *PLoS Negl Trop Dis.* **7**, e2070.
- Hesham Al-Mekhlafi M, Surin J, Atiya AS, Ariffin WA, Mohammed Mahdy AK & Che Abdullah H (2008) Pattern and predictors of soil-transmitted helminth reinfection among aboriginal schoolchildren in rural Peninsular Malaysia. *Acta Trop.* **107**, 200-4.
- Hotez PJ, Molyneux DH, Fenwick A *et al.* (2007) Control of neglected tropical diseases. *N Engl J Med.* **357**, 1018-27.
- Ing R, Su Z, Scott ME & Koski KG (2000) Suppressed T helper 2 immunity and prolonged survival of a nematode parasite in protein-malnourished mice. *Proc Natl Acad Sci USA.* **97**, 7078-83.
- Jacobs DR, Jr. & Tapsell LC (2007) Food, not nutrients, is the fundamental unit in nutrition. *Nutr Rev.* **65**, 439-50.
- Jia TW, Melville S, Utzinger J, King CH & Zhou XN (2012) Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Negl Trop Dis.* **6**, e1621.
- Katona P & Katona-Apte J (2008) The interaction between nutrition and infection. *Clin Infect Dis.* **46**, 1582-8.
- Kau AL, Ahern PP, Griffin NW, Goodman AL & Gordon JI (2011) Human nutrition, the gut microbiome and the immune system. *Nature.* **474**, 327-36.
- Keiser J & Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. *Adv Parasitol.* **73**, 197-230.
- Keusch GT (2003) The history of nutrition: malnutrition, infection and immunity. *J Nutr.* **133**, 336S-340S.
- Kightlinger LK, Seed JR & Kightlinger MB (1996) *Ascaris lumbricoides* aggregation in relation to child growth status, delayed cutaneous hypersensitivity, and plant anthelmintic use in Madagascar. *J Parasitol.* **82**, 25-33.
- Knopp S, Mohammed KA, Speich B *et al.* (2010) Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial. *Clin Infect Dis.* **51**, 1420-8.
- Koski KG & Scott ME (2001) Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. *Annu Rev Nutr.* **21**, 297-321.
- Long KZ, Estrada-Garcia T, Rosado JL *et al.* (2006) The effect of vitamin A supplementation on the intestinal immune response in Mexican children is modified by pathogen infections and diarrhea. *J Nutr.* **136**, 1365-70.

- Long KZ, Rosado JL, Montoya Y *et al.* (2007) Effect of vitamin A and zinc supplementation on gastrointestinal parasitic infections among Mexican children. *Pediatrics*. **120**, e846-55.
- Maggini S, Wenzlaff S & Hornig D (2010) Essential role of vitamin C and zinc in child immunity and health. *J Int Med Res*. **38**, 386-414.
- Moher D, Liberati A, Tetzlaff J & Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. **6**, e1000097.
- Mupfasoni D, Karibushi B, Koukounari A *et al.* (2009) Polyparasite helminth infections and their association to anaemia and undernutrition in Northern Rwanda. *PLoS Negl Trop Dis*. **3**, e517.
- Murray CJL, Vos T, Lozano R *et al.* (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. **380**, 2197-223.
- Nchito M, Geissler PW, Mubila L, Friis H & Olsen A (2009) The effect of iron and multi-micronutrient supplementation on *Ascaris lumbricoides* reinfection among Zambian schoolchildren. *Trans R Soc Trop Med Hyg*. **103**, 229-36.
- Nga TT, Winichagoon P, Dijkhuizen MA *et al.* (2009) Multi-micronutrient-fortified biscuits decreased prevalence of anemia and improved micronutrient status and effectiveness of deworming in rural Vietnamese school children. *J Nutr*. **139**, 1013-21.
- Nga TT, Winichagoon P, Dijkhuizen MA, Khan NC, Wasantwisut E & Wieringa FT (2011) Decreased parasite load and improved cognitive outcomes caused by deworming and consumption of multi-micronutrient fortified biscuits in rural Vietnamese schoolchildren. *Am J Trop Med Hyg*. **85**, 333-40.
- Olsen A (2007) Efficacy and safety of drug combinations in the treatment of schistosomiasis, soil-transmitted helminthiasis, lymphatic filariasis and onchocerciasis. *Trans R Soc Trop Med Hyg*. **101**, 747-58.
- Olsen A, Nawiri J & Friis H (2000) The impact of iron supplementation on reinfection with intestinal helminths and *Schistosoma mansoni* in western Kenya. *Trans R Soc Trop Med Hyg*. **94**, 493-9.
- Olsen A, Thiong'o FW, Ouma JH *et al.* (2003) Effects of multimicronutrient supplementation on helminth reinfection: a randomized, controlled trial in Kenyan schoolchildren. *Trans R Soc Trop Med Hyg*. **97**, 109-14.
- Payne LG, Koski KG, Ortega-Barria E & Scott ME (2007) Benefit of vitamin A supplementation on ascaris reinfection is less evident in stunted children. *J Nutr*. **137**, 1455-9.
- Pullan RL & Brooker SJ (2012) The global limits and population at risk of soil-transmitted helminth infections in 2010. *Parasit Vectors*. **5**, 81.

- Saldiva SR, Carvalho HB, Castilho VP, Struchiner CJ & Massad E (2002) Malnutrition and susceptibility to enteroparasites: reinfection rates after mass chemotherapy. *Paediatr Perinat Epidemiol.* **16**, 166-71.
- Scrimshaw NS & SanGiovanni JP (1997) Synergism of nutrition, infection, and immunity: an overview. *Am J Clin Nutr.* **66**, 464S-477S.
- Scrimshaw NS, Taylor CE & Gordon JE (1959) Interactions of nutrition and infection. *Am J Med Sci.* **237**, 367-403.
- Steinmann P, Utzinger J, Du ZW *et al.* (2011) Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp.: a randomized controlled trial. *PLoS One.* **6**, e25003.
- Stephenson LS, Latham MC & Ottesen EA (2000) Malnutrition and parasitic helminth infections. *Parasitology.* **121 Suppl**, S23-38.
- Stratton RJ & Elia M (2010) Encouraging appropriate, evidence-based use of oral nutritional supplements. *Proc Nutr Soc.* **69**, 477-87.
- Tomkins A & Watson F (1989) Malnutrition and infection - a review. Geneva, United Nations.
- Tripathy K, Garcia FT & Lotero H (1971) Effect of nutritional repletion on human hookworm infection. *Am J Trop Med Hyg.* **20**, 219-23.
- UN (2010) The Millennium Development Goals Report 2010. New York, United Nations.
- Utzinger J & Keiser J (2004) Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. *Expert Opin Pharmacother.* **5**, 263-85.
- WHO (2012) Eliminating soil-transmitted helminthiasis as a public health problem in children. Geneva, World Health Organization.
- Yap P, Du ZW, Wu FW *et al.* (2013) Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in Yunnan, People's Republic of China. *Am J Trop Med Hyg.* **89**, 23-31.
- Zhou H, Watanabe C & Ohtsuka R (2007) Impacts of dietary intake and helminth infection on diversity in growth among schoolchildren in rural south China: a four-year longitudinal study. *Am J Hum Biol.* **19**, 96-106.
- Ziegelbauer K, Speich B, Mäusezahl D, Bos R, Keiser J & Utzinger J (2012) Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. *PLoS Med.* **9**, e1001162.

12.9. Appendix

Supplementary table 12.1. Detailed search strategies for this systematic review.

Databases	Search sets
1) Pubmed/Medline 2) Embase 3) Cochrane Library 4) Cochrane Central Register of Controlled Trials 5) Virtual Health Library	<ul style="list-style-type: none"> ▪ Reinfection AND nutrition ▪ Reinfection AND undernutrition ▪ Reinfection AND malnutrition ▪ Reinfection AND iron ▪ Reinfection AND zinc ▪ Reinfection AND vitamin ▪ Reinfection AND nutritional supplementation ▪ Multiparasitism AND nutrition ▪ Polyparasitism AND nutrition ▪ Infection intensity AND nutrition ▪ Soil-transmitted helminths AND nutrition ▪ Soil-transmitted helminths AND reinfection ▪ Soil-transmitted helminths AND undernutrition ▪ Soil-transmitted helminths AND micronutrient supplementation ▪ Hookworm AND nutritional supplementation ▪ <i>Trichuris</i> AND nutritional supplementation ▪ <i>Ascaris</i> AND nutritional supplementation
6) Science Direct	All the above except: <ul style="list-style-type: none"> ▪ Infection intensity AND nutrition
7) VIP Information 8) China National Knowledge Infrastructure	<ul style="list-style-type: none"> ▪ 土源性蠕虫 (Soil-transmitted helminths) ▪ 蛔虫感染 (Ascariasis) ▪ 鞭虫感染 (Trichuriasis) ▪ 钩虫感染 (Ancylostomiasis)

Supplementary table 12.2. List of the 8 studies excluded from this systematic review.

Study	Reasons for exclusion
Ahmann <i>et al.</i> 1933	Case series of 3 individuals
Bundy <i>et al.</i> 1987	A review on the mechanisms of interactions between helminths and host malnutrition
Figaro-Fletcher <i>et al.</i> 1988	Unable to obtain full text; only the abstract was available
Quihui-Cota <i>et al.</i> 2004	A cross-sectional study which is unable to demonstrate causality
Hughes <i>et al.</i> 2006	Focuses on general interactions between malnutrition and immune impairment without being specific for soil-transmitted helminth infection
Koski <i>et al.</i> 2001	A review on the effects of nutritional deficiencies on gastrointestinal nematodes of humans, livestock and laboratory rodents
Neumann <i>et al.</i> 1975	Focuses on general immunologic responses in malnourished children without being specific for soil-transmitted helminth infection
Tripathy <i>et al.</i> 1971	Case series of 12 individuals

Supplementary table 12.3. Detailed summary of primary and secondary outcomes of the 15 studies retained for inclusion in the systematic review

Study	Outcomes
1) Nga <i>et al.</i> 2011	<p>Primary: i) Significant^a difference in infection intensity (EPG) reduction observed for <i>A. lumbricoides</i> after 4 months <i>Differences in infection intensity between 4 months and baseline for children taking 'fortified biscuits and albendazole' versus 'albendazole alone': for A. lumbricoides: -7,728 EPG versus -4,656 EPG. For T. trichiura: -72 EPG versus -48 EPG and for hookworm: -672 EPG versus -552 EPG, but differences for T. trichiura and hookworm were not statistically significant.</i></p> <p>Secondary: Children receiving fortified biscuits i) had their mid-upper arm circumference slightly improved (+0.082 cm) and ii) scored higher (+0.34) on the digit span forward cognitive test. These improvements were statistically significant</p>
2) Nga <i>et al.</i> 2009	<p>Primary: i) Significant difference in prevalence reduction observed for <i>A. lumbricoides</i> and <i>T. trichiura</i> after 4 months <i>Differences in prevalence between 4 months and baseline for children taking 'fortified biscuits and albendazole' versus 'albendazole alone': for A. lumbricoides: -25% versus -9% and for T. trichiura: -19% versus -4%. For hookworm: -2% versus 0% but differences for hookworm were not statistically significant</i></p> <p>Secondary: Fortified biscuits significantly reduced the odds of i) anaemia and ii) deficiencies of zinc and iodine by 44%, 48% and 47%, respectively</p>
3) Nchito <i>et al.</i> 2009	<p>Primary: i) No significant difference in re-infection rate and infection intensity of <i>A. lumbricoides</i> between the different intervention groups <i>Differences in prevalence between 6 months and baseline: -21% in 'iron only' versus -18% in 'multi-micronutrients only', -12% in 'placebo' and -6% in 'iron with multi-micronutrients'. In all intervention arms, prevalences were back to baseline levels at 10 months</i> <i>Differences in infection intensity as compared to baseline: at 6 months, -1,726 EPG in 'iron only' versus -1,369 EPG in 'iron with multi-micronutrients', +1,501 EPG in 'multi-micronutrients only' and +380 EPG in 'placebo' At 10 months, -1,233 EPG in 'iron only' versus -9 EPG in 'placebo', +138 EPG in 'iron with multi-micronutrients' and +983 EPG in 'multi-micronutrients only'</i></p> <p>ii) There was a significant interaction between iron and multi-micronutrient supplementation on re-infection rate at 6 months <i>Re-infection rate: 22% in 'iron taken without multi-micronutrients' versus 38% in 'iron taken with multi-micronutrients'</i></p> <p>iii) A significant dose-response relationship between the number of iron tablets taken and the reduction in infection intensity</p>

	was observed at 6 months. Children who took iron were re-infected with 50% of the infection intensity found in the placebo group. In a sub-group analysis, children who took more than 50 iron tablets were re-infected with 20% of the infection intensity found in children who took placebo tablets
4) Long <i>et al.</i> 2007	<p>Primary: i) ‘Zinc alone’ significantly increased the prevalence of <i>A. lumbricoides</i> by 51% as compared to placebo. ‘Vitamin A with zinc’ decreased the prevalence by 1% and ‘vitamin A alone’ increased the prevalence by 18% as compared to placebo. Both changes were not statistically significant</p> <p>Secondary: i) A combination of vitamin A and zinc significantly reduced the mean duration of <i>A. lumbricoides</i> infection (3.6 days) as compared to placebo (5.5 days)</p> <p>ii) A combination of vitamin A and zinc significantly reduced the incidence of <i>A. lumbricoides</i>-associated diarrhoea by 73%</p>
5) Long <i>et al.</i> 2006	Secondary: i) In the event of an <i>A. lumbricoides</i> infection, vitamin A supplemented children had increased interleukin 4 (IL-4) levels compared to un-supplemented children (odds ratio = 12.06)
6) Olsen <i>et al.</i> 2003	<p>Primary: i) No significant difference in re-infection rate and infection intensity throughout the 11 months</p> <p><i>Differences in prevalence between 11 months and baseline for children taking ‘multi-micronutrients’ versus ‘placebo’:</i> for <i>A. lumbricoides</i>: -1% versus -0.2%; for <i>T. trichiura</i>: -26% versus -27%; for hookworm: -37% versus -35%</p> <p><i>Differences in infection intensity between 11 months and baseline for children taking ‘multi-micronutrients’ versus ‘placebo’:</i> for <i>A. lumbricoides</i>: -0.5 EPG versus -0.3 EPG; for <i>T. trichiura</i>: -4 EPG for both groups; for hookworm: -7 EPG versus -6 EPG</p>
7) Olsen <i>et al.</i> 2000	<p>Primary: i) No significant difference in re-infection rate and infection intensity in children after 12 months.</p> <p><i>Differences in prevalence between 12 months and baseline for children taking ‘iron’ versus ‘placebo’:</i> for <i>A. lumbricoides</i>: +15% for both groups; for <i>T. trichiura</i>: -11% versus -6%; for hookworm: -30% versus -25%</p> <p><i>Differences in infection intensity between 12 months and baseline for children taking ‘iron’ versus ‘placebo’:</i> for <i>A. lumbricoides</i>: +1,115 EPG versus -1,710 EPG; for <i>T. trichiura</i>: +13 EPG versus -15 EPG; for hookworm: -10 EPG versus +10 EPG</p> <p>ii) Significant difference in re-infection rate observed in adults at 4 or 12 months</p> <p><i>Differences in prevalence as compared to baseline for adults taking ‘iron’ versus ‘placebo’:</i> at 4 months for hookworm: -61% versus -67%. Re-infection rates for <i>A. lumbricoides</i> and <i>T. trichiura</i> at 4 months were not reported. At 12 months, for <i>A. lumbricoides</i>: + 9% versus +26%; for <i>T. trichiura</i>: -11% versus + 2%; for hookworm: -37% for both groups but not statistically significant</p> <p>iii) No significant difference in infection intensity in adults at 12 months.</p> <p><i>Differences in infection intensity between 12 months and baseline for adults taking ‘iron’ versus ‘placebo’:</i> for <i>A. lumbricoides</i>: - 1,085 EPG versus -3,795 EPG; for <i>T. trichiura</i>: +8 EPG versus +5 EPG; for hookworm: -45 EPG versus +58 EPG</p>

8) Grazioso <i>et al.</i> 1993	<p>Primary: i) No significant difference in re-infection rate and infection intensity at the end of 120-150 days <i>Difference in prevalence between end of 120-150 days and baseline for children taking 'zinc' versus 'placebo': -65% versus -66%. Specific prevalences of <i>A. lumbricoides</i> and <i>T. trichiura</i> after the mebendazole therapy were not mentioned. Actual values of infection intensity were also not reported</i></p>
9) Halpenny <i>et al.</i> 2013	<p>Primary: i) Children with higher height-for-age (HAZ) score have hookworm infection intensity 0.49 times that of their peers with lower HAZ score at the end of cycle 2. This was statistically significant ii) Children with higher HAZ score have <i>A. lumbricoides</i> infection intensity 0.15 times that of their peers with lower HAZ score at the end of cycle 1. This was statistically significant</p>
10) Hesham Al-Mekhlafi <i>et al.</i> 2008	<p>Primary: i) Three months after de-worming with albendazole, stunted children had a higher re-infection rate (61%) with soil-transmitted helminths (stratification of species not done) than non-stunted children (40%) only in the univariate analysis. This significant difference was lost in the multivariate analysis. Also at 3 months, children with underweight <i>versus</i> non-underweight have re-infection rates of 51% <i>versus</i> 43% but this was not statistically significant <i>Re-infection rates at 6 months:</i> stunted children <i>versus</i> non-stunted children were 88% <i>versus</i> 73%, while underweight <i>versus</i> non-underweight were 86% <i>versus</i> 75%. However, these observations were not statistically significant</p>
11) Payne <i>et al.</i> 2007	<p>Primary: i) Vitamin A supplemented children had significantly lower (-3.6 EPG) infection intensity at 3 months as compared to non-supplemented ones. However, infection intensity for supplemented children was already significantly lower (-6.3 EPG) at baseline ii) Stunted children had significantly higher <i>A. lumbricoides</i> infection intensity than non-stunted at both 3 (+1.6 EPG) and 5 (+2.0 EPG) months, regardless of vitamin A supplementation iii) In non-stunted children, prevalence and infection intensity of <i>A. lumbricoides</i> were significantly lower in supplemented children (13% and 1 EPG) than non-supplemented ones (45% and 32 EPG) at 3 months. At 5 months, the differences were 50% and 54 EPG <i>versus</i> 55% and 147 EPG but they were not statistically significant anymore iv) In stunted children, prevalence and infection intensity of <i>A. lumbricoides</i> between supplemented and non-supplemented ones were not statistically significant at 3 and 5 months. At 3 months: 38% and 19 EPG <i>versus</i> 50% and 39 EPG. At 5 months, 73% and 544 EPG <i>versus</i> 70% and 102 EPG v) In stunted children, infection intensity of <i>A. lumbricoides</i> at 3 months was significantly lower (6 EPG) in children who received vitamin A within 6 weeks of de-worming as compared to children who received vitamin A between 6 and 12 weeks before de-worming (122 EPG) vi) The 3-month re-infection rate of <i>A. lumbricoides</i> increased significantly as the interval between supplementation and de-</p>

	worming increased. Exact figures are not reported
12) Saldiva <i>et al.</i> 2002	Primary: i) After 1 year of follow-up, 38% of the undernourished children were re-infected with helminths, in particular <i>A. lumbricoides</i> and <i>T. trichiura</i> , while only 25% of eutrophic children were re-infected. However, this statistically significant difference was lost once maternal literacy and per capita incoming rate were controlled for
13) Hagel <i>et al.</i> 1999	Primary: i) At 8 months after the end of the 12-month treatment period, children $\leq 10^{\text{th}}$ percentile for height and weight/age (68% and 87%, respectively) showed significantly higher re-infection rates with <i>A. lumbricoides</i> than children $> 10^{\text{th}}$ percentile (32% and 13%, respectively). However, this observation could be confounded by socio-economic factors (not accounted for in the analysis), as there were a significantly higher proportion of children at or below the 10 th percentile for height or weight/age in extreme poverty as compared to children in critical poverty
14) Kightlinger <i>et al.</i> 1996	Primary: i) After 12 months, the best-nourished children had lower <i>A. lumbricoides</i> egg counts than children with reduced growth indicators, but these differences were not statistically significant <i>Egg counts for best-nourished versus under-nourished children:</i> in terms of weight-for-age, 1,995 EPG <i>versus</i> 3,162-3,981 EPG and in terms of height-for-age, 3,162 EPG <i>versus</i> 3,981–5,012 EPG iii) Difference in worm burden (geometric mean number of worms per child) among children with normal growth (12.5) <i>versus</i> stunted and underweight (11.5) <i>versus</i> stunted, underweight and wasted (16.0) was not significant.
15) Hagel <i>et al.</i> 1995	Secondary: i) No significant change in anti-<i>Ascaris</i> IgE levels was observed in undernourished children, while levels in well-nourished children increased significantly <i>Differences between 12 months and baseline for under-nourished versus well-nourished:</i> in terms of weight-for-age, -0.10 pru/mL <i>versus</i> +0.55 pru/mL; in terms of height-for-age, -0.05 pru/mL <i>versus</i> +0.65 pru/mL

^a “Significant” indicates statistical significance when an outcome measure has a *P*-value <0.05.

13. Discussion

The research performed within the frame of this Ph.D. thesis focuses on gaining a deeper understanding of the epidemiology and burden of soil-transmitted helminth infections among children of various Bulang communities in a mountainous region of southwest Yunnan province, P.R. China. Building upon a longstanding and productive collaboration with our Chinese partners in Shanghai and Yunnan (Yuan *et al.* 2000; Xiao *et al.* 2002; Zhou *et al.* 2005; Steinmann *et al.* 2007, 2008), the studies described in this thesis sought to widen the scope of helminthic research in the region, particularly on the subtle burden of soil-transmitted helminthiasis. When stratified according to the strategic axes guiding the work of Swiss TPH, an emphasis on innovation and validation was noted (Figure 13.1). Importantly, the work benefitted from an in-house cross-unit collaboration, where vast expertise and experiences on all aspects of helminthiasis (Ecosystem Health Sciences unit) and physical activity and fitness (Chronic Disease Epidemiology unit) were combined.

With the hypothesis that a reduction in physical fitness could result from chronic soil-transmitted helminth infections, we first conducted a cross-sectional survey to explore the associations of these infections with physical fitness and assess the suitability of two popular physical fitness tests, namely the Harvard step test and the 20-m shuttle run test, in a mountainous and resource-constrained setting (Yap *et al.* 2012). To examine the reduced physical fitness observed with *T. trichiura* infections in the first study, a randomised controlled trial was conducted where an array of physical fitness and anthropometric measurements were performed. Changes in these indicators, due to treatment, were monitored over a 6-month period (Yap *et al.* 2013c). Finally, by extrapolating the results from the trial, we attempted a first prediction and visualization of the change in physical fitness due to soil-transmitted helminth infections across P.R. China. During the course of this thesis, re-infection dynamics of soil-transmitted helminths following albendazole treatment were also studied (Yap *et al.* 2013a) and evidence on the influence of nutrition status on re-infection with soil-transmitted helminths were systematically reviewed (Yap *et al.* 2013b). In the rest of this chapter, major observations arising from the aforementioned research, along with their public health implications, will be discussed.

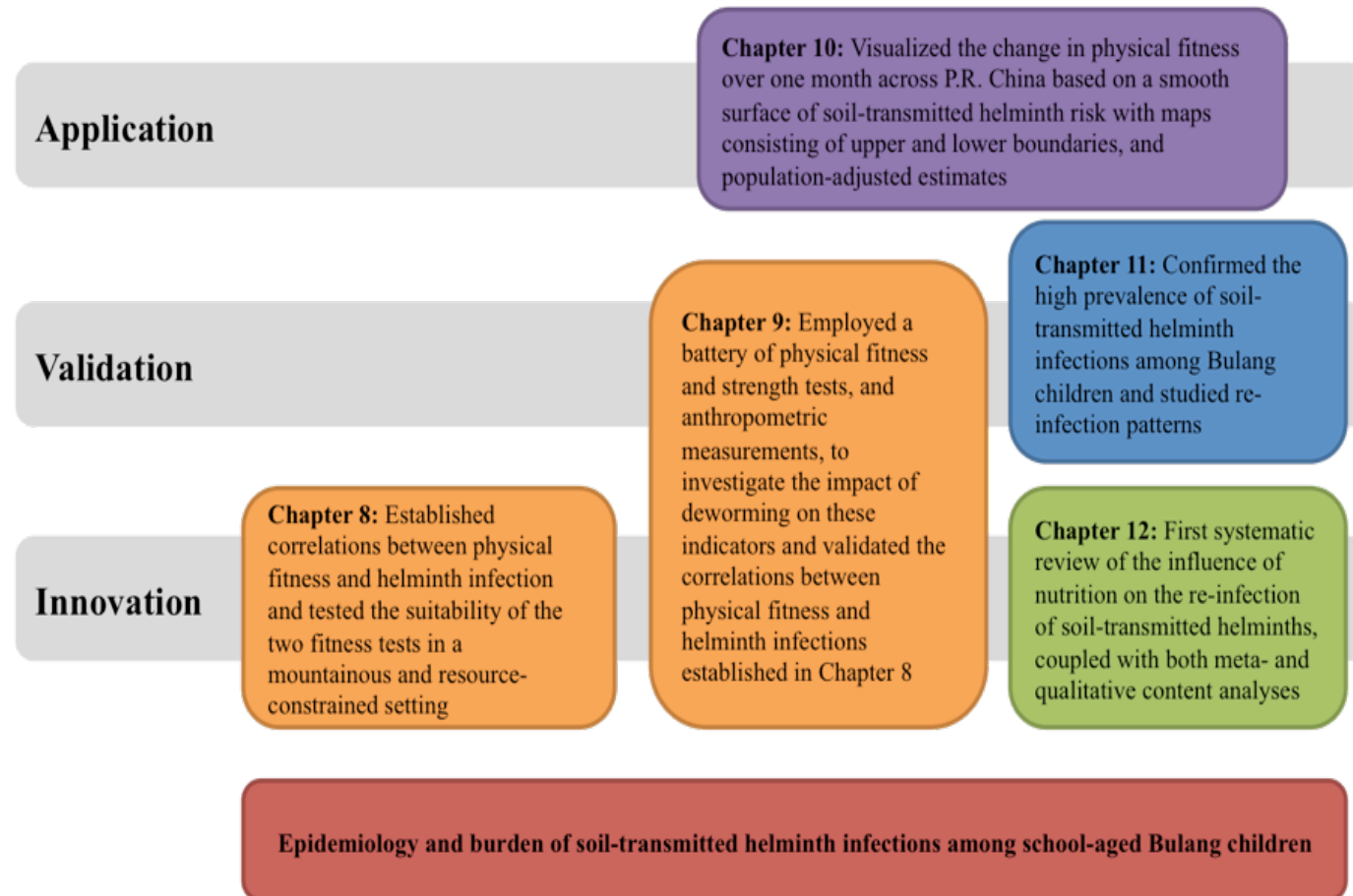


Figure 13.1. An overview of the described studies, stratified according to the strategic axes guiding the research work of Swiss TPH.

13.1. Burden of soil-transmitted helminthiasis with a focus on physical fitness

Physical fitness is important for the development of a child, as it has been correlated with increased self-esteem, cognitive skills and academic performance (Castelli *et al.* 2007; Niederer *et al.* 2011; Vedul-Kjelsås *et al.* 2012). In impoverished communities, where proper sanitation and clean water are often missing, the presence of soil-transmitted helminthiasis has been suggested to reduce physical fitness in children through diminished growth and anaemia-induced fatigue. However, population-based evidence supporting this proposition has been reported in only three studies from Kenya (Stephenson *et al.* 1990, 1993; Bustinduy *et al.* 2011), and this dearth of evidence has prompted the investigations presented in this thesis.

To assess physical fitness in children, a myriad of tests could be employed (Artero *et al.* 2011). Based on the physical fitness research of our colleagues in Côte d'Ivoire (Müller *et al.* 2011), our pilot cross-sectional survey in P.R. China demonstrated the good technical and operational feasibility of the 20-m shuttle run test (Léger *et al.* 1988), which measures cardiovascular endurance, in a rural and mountainous region under resource-constrained settings. Running was an easy concept for the children to grasp, but effort still had to be made to get them to follow the pace of the pre-recorded sound signals. In contrast, it was difficult to standardize the Harvard Step Test (Gallagher & Brouha 1943) across the different villages and children took a longer time to learn and perform the test properly. As physical fitness is not solely determined by cardiovascular endurance, additional tests for musculoskeletal strength were added in a following study. Both the grip strength (España-Romero *et al.* 2008) and standing broad jump (TopEndSports 2013) tests were easy to set up as only a dynamometer, for the former, and a flat surface of about 2-m long and a measuring tape, for the latter, were needed. Similar to the 20-m shuttle run test, performing the grip strength test turned out to be as intuitive for the children. On the other hand, the standing broad jump test required more explanation and training. To the best of our knowledge, the usage of a battery of physical fitness tests in children to assess a particular component of the burden of soil-transmitted helminth infections is a novel concept. Our experiences in the field highlighted that a spectrum of tests is necessary as not all children excelled in one particular test. More importantly, since the impact of soil-transmitted helminthiasis on physical fitness is

uncertain, increasing the diversity of tests will allow a more comprehensive inquiry on the subtleties of effects.

In our studies, we first found significant associations between *T. trichiura* infections and performance in the 20-m shuttle run test in a cross-sectional survey. After adjusting for age and sex, the VO_2 max estimate of children infected with *T. trichiura* was $1.9 \text{ ml kg}^{-1} \text{ min}^{-1}$ lower than that of their non-infected peers. Until exhaustion, they also completed six 20-m laps less (Yap *et al.* 2012). During the subsequent randomised controlled trial, we found that in the follow-ups, *T. trichiura*-infected children had $1.6 \text{ ml kg}^{-1} \text{ min}^{-1}$ less increase in their VO_2 max estimate from baseline than their non-infected counterparts. Consequently, the increase in the number of 20-m laps completed from baseline was between four and six laps less. In addition, when stratified according to their longitudinal infection intensity patterns, children with no or minimal *T. trichiura* and hookworm re-infection at follow-ups had significantly more increase in their VO_2 max estimate and the number of 20-m laps completed from baseline than peers with high infection intensity of all soil-transmitted helminth species (Yap *et al.* 2013c). The negative impacts of light to moderate *T. trichiura* infections on physical fitness consistently observed in both studies are intriguing and unexpected, particularly because *T. trichiura* is conventionally deemed the least harmful soil-transmitted helminth species as there is no visceral migration of the larvae unlike that observed for *A. lumbricoides* and hookworm (Brooker 2010). However, the invasive mechanisms adult *T. trichiura* worms use to anchor their whip-like anterior end into the large intestine and caecum, can cause abdominal pain in the short term, and rectal prolapse in the long term, especially when the worm burden is high (Hall *et al.* 2008). These pathological features in the intestine have been shown to result in malnutrition, stunting, anaemia, and finger-clubbing (Bundy & Cooper 1989), and could arguably cause the physical fitness reduction observed in *T. trichiura*-infected children enrolled in our studies.

The impaired physical fitness due to *T. trichiura* infections observed in the work of this thesis highlight that the current burden estimate of soil-transmitted helminth infections, in particular *T. trichiura* infections, might be underestimated and there are still subtle and hidden morbidities to be revealed and quantified. Within our group at Swiss TPH, other tools, such as quality-of-life (QoL) questionnaires and cognitive tests (Ziegelbauer *et al.* 2010; Furst *et al.* 2012; Hürlimann 2013), have

been used in attempts to better understand the burden of soil-transmitted helminthiasis. Interestingly, a community-level cross-sectional survey employing QoL questionnaires in Côte d'Ivoire found a 13-point reduction in the QoL of people infected with *T. trichiura* (Fürst *et al.* 2012), while a school-level cross-sectional survey in P.R. China did not find any association between QoL and soil-transmitted helminth infections (Ziegelbauer *et al.* 2010). This shows that although the use of different tools to explore the varied facets of the disease burden from a patient's perspective is necessary, many of these tools remain to be validated on a wider scale among different cultural backgrounds, as the local eco-epidemiological-cultural setting of the patient is likely to be a confounder of disease impact (King & Bertino 2008). Furthermore, more sensitive tools are needed to detect soil-transmitted helminth infections of light intensity (Bergquist *et al.* 2009; Knopp *et al.* 2008) and the subtle morbidities associated with them.

For a neglected tropical disease such as soil-transmitted helminthiasis, where individuals harbouring these parasitic worms do not present themselves with distinct symptoms, the determination of an average disability weight for the quantification of the burden of soil-transmitted helminthiasis using DALYs can be problematic (Salomon *et al.* 2012). When one imagines a child infected with soil-transmitted helminths, it is difficult to conjure a scene where the child is suffering from immediate pain and showing signs of debilitation, and assign a high disability weight to the disease, even though chronic and subtle morbidities can have significant consequences in their future health and development (Hotez *et al.* 2008). In developing countries with limited resources and highly endemic with soil-transmitted helminths, such an underestimation of the burden can cause control of these infections to take a backseat. Therefore, it is also necessary to devise a method to combine and incorporate field-based evidence into the calculation of DALYs, as they are widely used by health-policy makers and their funding partners today as a primary currency to prioritize national and international investments in disease control (King & Bertino 2008; Murray *et al.* 2012).

Summarising the challenges that lie ahead in the estimation of the burden of soil-transmitted helminth infections, we need a variety of culturally validated and sensitive tests and tools, which can provide an objective assessment of subtle morbidities from a patient's perspective. As the level of morbidity is purported to be

infection intensity-dependent (WHO 2012), studies should also take into account both infection intensity and multiparasitism when reporting outcome measures. Innovative ways to obtain, present and interpret such data, using three-dimensional graphics and cluster analysis, have been demonstrated in an earlier chapter of this thesis (Yap *et al.* 2013c). Besides establishing field-based evidence, an algorithm that combines the results of all the different tests into a standardised summary estimate is also essential. Building such a concrete base of evidence will help to achieve a more accurate reappraisal of the disability weights assigned to soil-transmitted helminthiasis, particularly for *T. trichiura* infections.

13.2. Nutrition status and soil-transmitted helminth infections

The occurrences of poor host nutrition status and soil-transmitted helminth infections share not only similar geographical locations (FAO 2012; Pullan & Brooker 2012), but also the same pathological spot, namely the intestines, in the human body. Many population-based studies have established that soil-transmitted helminth infections could lead to malnutrition and impaired growth in children (Stephenson *et al.* 2000; Crompton & Nesheim 2002; Zhou *et al.* 2007; Hall *et al.* 2008). Based on these observations, it has been assumed that removing the parasitic worms alone will reverse the retardation of growth and improve host nutrition status, and thus, many studies have tried to determine the impact of deworming on the nutrition status of children. A Cochrane review concluded that there is currently no sufficient high quality evidence to show that deworming programmes have benefits on the nutrition and haemoglobin levels in children (Taylor-Robinson *et al.* 2012). In a recent randomised controlled trial from India (Awasthi *et al.* 2013), no statistically significant difference in anthropometric measurements was detected between the albendazole-treated and control groups, consisting of 1 million children, aged 1- to 6-year-old at baseline. In this thesis, significant beneficial effects of deworming on anthropometric measurements and haemoglobin levels were also not detected (Yap *et al.* 2013c). These results might seem counterintuitive, especially when a major burden of soil-transmitted helminthiasis is nutrient deficiency, and have led to doubts on the benefits of routine mass deworming (Nagpal *et al.* 2013). However, it is important to realize that children harbouring chronic intestinal infections and living in unhygienic conditions often suffer from enteropathies, characterized by intestinal inflammation,

diminished absorptive capacity, and elevated intestinal permeability (Prendergast & Kelly 2012). These symptoms can result in chronic undernutrition indicated by the presence of stunting. Stunting occurs in about 32% of children living in developing countries (Black *et al.* 2008) and certainly in the majority of the Bulang children enrolled in our studies. It develops between -9 months (fetal life) and +24 months (neonatal life), and its effects are irreversible (Black *et al.* 2008). Likewise for anaemia, enteropathies might cause it to be a permanent problem early in children from developing countries (DeMaeyer & Adiels-Tegman 1985). In addition, administering an anthelmintic tablet to a child is not equivalent to providing a nutritious meal, the former is simply a drug indicated for the treatment of soil-transmitted helminth infections and the latter is what is required for a child to grow. Therefore, studies measuring the impact of deworming alone on nutrition status in children might have to consider enrolling children from an earlier age group and providing sufficient nutrients and time for the child to catch up on their growth, before denying the health benefits that anthelmintic drugs potentially have. Since soil-transmitted helminths are not the only parasites that can lead to enteropathies, studies, which assess the collective occurrence of bacterial, intestinal protozoal and helminth infections and evaluate interventions for their control, might also be more useful in determining the impact of deworming on nutrition status.

The relationship between nutrition status and soil-transmitted helminth infections has another dimension, and that is the influence of nutrition on the susceptibility to soil-transmitted helminthiasis. Unfortunately, there is a scarcity of studies evaluating this issue and contemporary evidence are inconclusive (Koski & Scott 2001). To summarise the available evidence on influence of the natural nutrition status of the host and nutritional supplementation on re-infection with soil-transmitted helminths, a systematic review, coupled with meta- and qualitative content analyses, was performed during the course of this thesis (Yap *et al.* 2013b). Among the various nutritional supplementation interventions reviewed, multi-micronutrients seemed to have the clearest effect with regards to lowering re-infection rates and intensity of soil-transmitted helminths, whereas consumption of zinc or vitamin A alone might have a negative impact on these two outcomes measures. With regards to the natural nutrition status of the host, the general trend observed was that individuals with poor nutrition status suffered higher re-infection rates and intensities when compared to

their well-nourished peers. Several shortcomings in the included studies were also noted. First, none of the studies investigated the effect of nutritious whole foods as an intervention, which might have a distinct impact compared to synthetic supplements, since consumption of a broad range of nutrients from their natural sources might aid in their absorption and assimilation into the body (Jacobs & Tapsell 2007; Jacobs *et al.* 2009). Making dietary improvements with whole foods derived from the natural environment of the community might also make the intervention more readily accepted locally and thus, more sustainable. Second, research on the identification of synergistic combinations of nutritional supplements is lacking. Third, it is unclear whether sufficient anthelmintic treatment, nutritional supplementation and time post-intervention have been allowed for the body to build and utilize a reliable source of nutrition, to strengthen the immune system, and eventually discourage the re-infections with soil-transmitted helminths. Overall, the evidence reviewed is limited and of low quality.

Given their geographical overlaps and how closely intertwined their pathological effects on each other are, it makes scientific sense to address both undernutrition and soil-transmitted helminthiasis concurrently. However, the strong political lobby for mass drug administration against soil-transmitted helminthiasis and other neglected tropical diseases (Spiegel *et al.* 2010), and the lack of high quality evidence have caused such a research need to remain controversial and unexplored. Before dismissing the public health potential of combining nutrition management with anthelmintic treatment, study designs, which are able to investigate the complexities of the relationship between nutrition status and soil-transmitted helminthiasis, are needed to evaluate their combined effectiveness in improving host nutrition status with deworming or reducing re-infection with soil-transmitted helminths. Due to the complicated interplay of the different interactions, randomised controlled trials might not always be possible or appropriate and therefore, increased acceptance in the value of evidence from non-randomized studies should also be encouraged within the scientific community.

13.3. Impact of re-infection dynamics on control with chemotherapy

Although efficacy studies of albendazole against soil-transmitted helminths are numerous (Keiser & Utzinger 2008; Vercruysse *et al.* 2011), few studies have

assessed post-treatment re-infection patterns as rigorously as presented in this thesis (Jia *et al.* 2012; Yap *et al.* 2013a). In this study, we observed good efficacy of albendazole against *A. lumbricoides*, but also rapid re-infection at 4 months post-treatment, with prevalence reaching 80% of the pre-treatment prevalence; a low cure rate of 20% and a moderate egg reduction rate of 89% against *T. trichiura* infections; and high efficacy of albendazole against hookworm and minimal re-infection detected over the 6-month follow-up period (Yap *et al.* 2013a). Based on these species-specific re-infection dynamics and other field experiences on soil-transmitted helminth infections in the same region (Steinmann 2013), the following implications for their control with preventive chemotherapy (WHO 2006; Albonico *et al.* 2008) in a highly endemic setting are noted.

For hookworm infections, both the prevalence and infection intensity can be greatly reduced and maintained with single-dose albendazole treatment given on a yearly basis. This suggests that continued chemotherapy might be able to reduce environmental contamination to a point that transmission is disrupted, leading to good control and possibly elimination of hookworm. In terms of *A. lumbricoides* infections, more regular chemotherapy cycles, such as single-dose albendazole twice a year, have to be implemented to counter the rapid re-infection. As regular treatment with albendazole alone might increase the chances of drug resistance development, the potential lack of sustainability in such a strategy highlights the need for the implementation of concurrent interventions (Prichard *et al.* 2013). With *T. trichiura* infections, single-dose albendazole treatment is unlikely to be efficacious in reducing infection intensity, which is the primary aim of preventive chemotherapy (Montresor *et al.* 2011; Vercruysse *et al.* 2011). At the moment, triple-dose albendazole treatment or combination drug therapy has been shown to be an alternative (Knopp *et al.* 2010; Steinmann *et al.* 2011). However, additional studies worldwide are required to monitor the efficacy of such intensive treatment regimen against this parasitic worm and the potential development of drug resistance (Diawara *et al.* 2013; Prichard *et al.* 2013). More importantly, new anthelmintics have to be developed for control of *T. trichiura* infections with preventive chemotherapy to be sustainable. Drugs, such as oxantel pamoate, are currently under clinical trials, and results from animal models have already shown promising trichuricidal properties (Keiser *et al.* 2013).

13.4. Integrated control of soil-transmitted helminthiasis in P.R. China

It is hoped that through a series of studies and literature reviews conducted within the framework of this Ph.D. thesis, a better understanding on the epidemiology and burden of soil-transmitted helminthiasis will help inform policies made on the control of these infections in P.R. China. Indeed, a national control programme against soil-transmitted helminth is overdue and urgently needed as P.R. China develops into a global powerhouse. Useful lessons can be learned from the control of schistosomiasis in the country for the past decades (Utzinger *et al.* 2005; Wang *et al.* 2008) and field evidence on the implementation of water, sanitation and hygiene promotion around the world (Bieri *et al.* 2013; Freeman *et al.* 2013; Greene *et al.* 2012; Gyorkos *et al.* 2013; Schmidlin *et al.* 2013; Steinmann 2013). First, by providing better definitions of the burden of soil-transmitted helminthiasis, we can create awareness for the disease and increase its public health significance within the community of health professionals. Efforts should also be devoted to communicating our findings with the general public and advocating for the rural and marginalized communities where soil-transmitted helminths are often endemic. The prediction and visualization exercise demonstrated in chapter 10 was an attempt to create materials that are easy to grasp and will capture the attention of a wider audience. In parallel, political will and commitment to control soil-transmitted helminth infections should also be sought. Second, in order for such a control programme to be comprehensive and sustainable, chemotherapy should be integrated with improved sanitation and water sources, which reduce environmental contamination, and health education, which inculcates good hygiene habits. It is also important for the control programme to have a certain level of flexibility so that the various interventions can be adapted to the local eco-epidemiological-cultural settings (Utzinger *et al.* 2009). Finally, there has to be enough resources and people from different disciplines involved to implement, monitor and evaluate the control program. For example, building capacity in local primary health care centers to diagnose and treat soil-transmitted helminth infections, getting sanitation engineers to be involved with latrine construction, and having health education and marketing specialists work with local teachers for hygiene promotion. Periodic follow-ups on these interventions will allow their effectiveness and acceptance by the local communities to be monitored and evaluated.

Although a vertically designed national control programme for soil-transmitted helminths might be less complicated and easier to execute, efforts should be made to combine it with control programmes of other diseases that have similar transmission pathways, diagnostic and treatment procedures, and intervention strategies. In the long term, such a strategy will make utilization of resources more efficient and increase the cost-effectiveness of control interventions. Following implementation, rigorous monitoring and evaluation protocols should be applied to determine the effectiveness and acceptance of the control interventions in endemic communities, and in the case of chemotherapy, to assess drug efficacy and the potential development of drug resistance.

13.5. Conclusions

In this thesis, we observed negative associations between *T. trichiura* infections and physical fitness among school-aged Bulang children in Yunnan province, P.R. China. This finding suggests that the current burden estimate of soil-transmitted helminth infections, in particular *T. trichiura* infections, might be underestimated and there are still subtle and hidden morbidities to be quantified. A paradigm shift is needed to further understand the burden of soil-transmitted helminth infections as the presence of co-infections and co-morbidities add layers of complexity to the task. A spectrum of culturally validated and sensitive tests and tools, which can provide an objective population-based assessment of subtle morbidities, has to be devised and outcome measures should be reported in relation to not just prevalence but also infection intensity, and in the context of multiparasitism, if present. Due to the interplay of the multi-faceted interactions between infection and morbidity, innovative study designs are needed and since randomised controlled trials might not always be possible or appropriate, increased acceptance in the value of evidence from non-randomized studies should be encouraged within the scientific community. An algorithm that combines the population-based evidence into a standardized summary estimate and visualization of the burden estimate with materials that can capture the attention of a wider audience are necessary. The importance of devoting efforts to communicate the burden of soil-transmitted helminth infections outside of our niche community of epidemiologists and public health specialists to the general public and policy makers is also essential, because when the burden is not well defined or communicated, control programmes against soil-transmitted helminthiasis will lose their credibility, making it difficult to garner public and political support to ensure their sustainability.

Despite the huge social and economic advances made by P.R. China over the past 40 years, the epidemiological findings on soil-transmitted helminthiasis from this thesis highlight that hotspots of this disease still exist, especially in the rural and marginalized communities. A national soil-transmitted helminth control programme is overdue and urgently needed as P.R. China further develops into a global powerhouse. In order to allow the utilization of resources to be more efficient and increase the cost-effectiveness of control interventions, efforts should be made to

combine it with control programmes of other neglected infectious diseases of poverty that have similar transmission pathways, diagnostic and treatment procedures and intervention strategies. With many of their rural communities starting to have their hands on the first rung of the development ladder, P.R. China seems to be in a good position to set a leading example on how to control and eliminate soil-transmitted helminthiasis, and possibly other neglected tropical diseases, for developing countries around the world

13.6. Identified research need

- Identify sensitive tools, which can provide objective assessments of subtle morbidities associated with soil-transmitted helminthiasis and other neglected tropical diseases, and validate them on a wider scale among different cultural backgrounds.
- Devise an algorithm that combines the results of all the different morbidity assessments into a standardised summary estimate, which can be incorporated into the calculation of DALYs, currently the primary currency used by health-policy makers and their funding partners to prioritize national and international investments in disease control.
- Rethink the multi-dimensional relationship between nutrition status and soil-transmitted helminth infections and investigate the combined effectiveness of nutrition management and anthelmintic treatment in improving host nutrition status with deworming or reducing re-infection with soil-transmitted helminths.
- Develop new anthelmintics or combination therapies for a more effective control of soil-transmitted helminthiasis, in particular *T. trichiura* infections, and monitor drug efficacy and the potential development of drug resistance.
- Develop and evaluate the effectiveness and acceptability of control interventions, against soil-transmitted helminth infections, which should be sensitive to the local eco-epidemiological-cultural settings.
- Gather preliminary data for the technical and operational feasibility of a national soil-transmitted helminth control programme in P.R. China.

13.7. Recommendation

- Given that the morbidities associated with soil-transmitted helminth infections is still a black box and more population-based evidence is needed to understand them, current reliance on DALYs predominately in developing health policies for soil-transmitted helminthiasis should be cautioned.
- Due to the complexities involved in understanding the epidemiology and burden of soil-transmitted helminthiasis, randomised controlled trials are not always possible or appropriate and therefore, increased acceptance in the value of evidence from non-randomized trial should be encouraged within the scientific community.
- Active communication of the burden of soil-transmitted helminth infections to the general public and policy makers should be encouraged in order to garner public and political support for control programmes against soil-transmitted helminthiasis.
- A national soil-transmitted helminth control programme needs to be in place and efforts should be made to combine it with control programmes of other neglected infectious diseases of poverty that have similar transmission pathways, diagnostic and treatment procedures, and intervention strategies.

13.8. References

- Albonico M, Allen H, Chitsulo L, Engels D, Gabrielli AF, Savioli L (2008) Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Negl Trop Dis* **2**, e126.
- Artero EG, España-Romero V, Castro-Piñero J, Ortega FB, Suni J, Castillo-Garzon MJ, Ruiz JR (2011) Reliability of field-based fitness tests in youth *Int J Sports Med* **32**, 159-169.
- Awasthi S, Peto R, Read S, Richards SM, Pande V, Bundy D (2013) Population deworming every 6 months with albendazole in 1 million pre-school children in north India: DEVTA, a cluster-randomised trial. *Lancet* **381**, 1478-1486.
- Bergquist R, Johansen MV, Utzinger J (2009) Diagnostic dilemmas in helminthology: what tools to use and when? *Trends Parasitol* **25**, 151-156.
- Bieri FA, Gray DJ, Williams GM, Raso G, Li YS, Yuan L, He Y, Li RS, Guo FY, Li SM, McManus DP (2013) Health-education package to prevent worm infections in Chinese schoolchildren. *N Engl J Med* **368**, 1603-1612.
- Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, Mathers C, Rivera J (2008) Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* **371**, 243-260.
- Brooker S (2010) Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers - a review. *Int J Parasitol* **40**, 1137-1144.
- Bundy DA, Cooper ES (1989) *Trichuris* and trichuriasis in humans. *Adv Parasitol* **28**, 107-173.
- Bustinduy AL, Thomas CL, Fiutem JJ, Parraga IM, Mungai PL, Muchiri EM, Mutuku F, Kitron U, King CH (2011) Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric. *PLoS Negl Trop Dis* **5**, e1213.
- Castelli DM, Hillman CH, Buck SM, Erwin HE (2007) Physical fitness and academic achievement in third- and fifth-grade students. *J Sport Exerc Psychol* **29**, 239-252.
- Crompton DWT, Nesheim MC (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr* **22**, 35-59.
- DeMaeyer E, Adiels-Tegman M (1985) The prevalence of anemia in the world. *World Health Stat Q Rep* **38**, 302-316.
- Diawara A, Halpenny CM, Churcher TS, Mwandawiro C, Kihara J, Kaplan RM, Streit TG, Idaghdour Y, Scott ME, Basanez MG, Prichard RK (2013) Association between response to albendazole treatment and beta-tubulin genotype frequencies in soil-transmitted helminths. *PLoS Negl Trop Dis* **7**, e2247.

- España-Romero V, Artero EG, Santalieu-Pasias AM, Gutierrez A, Castillo MJ, Ruiz JR (2008) Hand span influences optimal grip span in boys and girls aged 6 to 12 years. *J Hand Surg Am* **33**, 378-384.
- FAO (2012) Undernourishment around the world in 2012. Rome, Food and Agriculture Organization of the United Nations.
- Freeman MC, Clasen T, Brooker SJ, Akoko DO, Rheingans R (2013) The impact of a school-based hygiene, water quality and sanitation intervention on soil-transmitted helminth reinfection: a cluster-randomized trial. *Am J Trop Med Hyg*, Epub ahead of print.
- Fürst T, Silué KD, Ouattara M, N'Goran DN, Adiossan LG, N'Guessan Y, Zouzou F, Koné S, N'Goran EK, Utzinger J (2012) Schistosomiasis, soil-transmitted helminthiasis, and sociodemographic factors influence quality of life of adults in Cote d'Ivoire. *PLoS Negl Trop Dis* **6**, e1855.
- Gallagher JR, Brouha L (1943) A simple method of testing the physical fitness of boys. *Am Assn Health Phys Ed Rec Res Q* **14**, 23-31.
- Greene LE, Freeman MC, Akoko D, Saboori S, Moe C, Rheingans R (2012) Impact of a school-based hygiene promotion and sanitation intervention on pupil hand contamination in Western Kenya: a cluster randomized trial. *Am J Trop Med Hyg* **87**, 385-93.
- Gyorkos TW, Maheu-Giroux M, Blouin B, Casapia M (2013) Impact of health education on soil-transmitted helminth infections in schoolchildren of the peruvian Amazon: a cluster-randomized controlled trial. *PLoS Negl Trop Dis* **7**, e2397.
- Hall A, Hewitt G, Tuffrey V, de Silva N (2008) A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Matern Child Nutr* **4 Suppl 1**, 118-236.
- Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J (2008) Helminth infections: the great neglected tropical diseases. *J Clin Invest* **118**, 1311-1321.
- Hürlimann E (2013) Effects of concomitant helminth infections on physical fitness and cognitive abilities in schoolchildren in a malaria-hyperendemic setting of Côte d'Ivoire (Personal communication).
- Jacobs DR, Jr., Gross MD, Tapsell LC (2009) Food synergy: an operational concept for understanding nutrition. *Am J Clin Nutr* **89**, 1543S-1548S.
- Jacobs DR, Jr., Tapsell LC (2007) Food, not nutrients, is the fundamental unit in nutrition. *Nutr Rev* **65**, 439-450.
- Jia TW, Melville S, Utzinger J, King CH, Zhou XN (2012) Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Negl Trop Dis* **6**, e1621.

-
- Keiser J, Tritten L, Silbereisen A, Speich B, Adelfio R, Vargas M (2013) Activity of oxantel pamoate monotherapy and combination chemotherapy against *Trichuris muris* and hookworms: revival of an old drug. *PLoS Negl Trop Dis* **7**, e2119.
- Keiser J, Utzinger J (2008) Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* **299**, 1937-1948.
- King CH, Bertino AM (2008) Asymmetries of poverty: why global burden of disease valuations underestimate the burden of neglected tropical diseases. *PLoS Negl Trop Dis* **2**, e209.
- Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, Marti H, Utzinger J (2008) Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis* **2**, e331.
- Knopp S, Mohammed KA, Speich B, Hattendorf J, Khamis IS, Khamis AN, Stothard JR, Rollinson D, Marti H, Utzinger J (2010) Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura*: a randomized controlled trial. *Clin Infect Dis* **51**, 1420-1428.
- Koski KG, Scott ME (2001) Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. *Annu Rev Nutr* **21**, 297-321.
- Léger LA, Mercier D, Gadoury C, Lambert J (1988) The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci* **6**, 93-101.
- Montresor A, Engels D, Chitsulo L, Gabrielli A, Albonico M, Savioli L, Lammie P (2011) The appropriate indicator should be used to assess treatment failure in STH infections. *Am J Trop Med Hyg* **85**, 579-580.
- Müller I, Coulibaly JT, Fürst T, Knopp S, Hattendorf J, Krauth SJ, Stete K, Righetti AA, Glinz D, Yao AK, Pühse U, N'Goran E K, Utzinger J (2011) Effect of schistosomiasis and soil-transmitted helminth infections on physical fitness of school children in Côte d'Ivoire. *PLoS Negl Trop Dis* **5**, e1239.
- Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY *et al.* (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2197-2223.
- Nagpal S, Sinclair D, Garner P (2013) Has the NTD community neglected evidence-based policy? *PLoS Negl Trop Dis* **7**, e2238.
- Niederer I, Kriemler S, Gut J, Hartmann T, Schindler C, Barral J, Puder JJ (2011) Relationship of aerobic fitness and motor skills with memory and attention in preschoolers (Ballabeina): a cross-sectional and longitudinal study. *BMC Pediatr* **11**, 34.

- Prendergast A, Kelly P (2012) Enteropathies in the developing world: neglected effects on global health. *Am J Trop Med Hyg* **86**, 756-763.
- Prichard RK, Basáñez MG, Boatín BA, McCarthy JS, García HH, Yang GJ, Sripa B, Lustigman S (2013) A research agenda for helminth diseases of humans: intervention for control and elimination. *PLoS Negl Trop Dis* **6**, e1549.
- Pullan RL, Brooker SJ (2012) The global limits and population at risk of soil-transmitted helminth infections in 2010. *Parasit Vectors* **5**, 81.
- Salomon JA, Vos T, Hogan DR, Gagnon M, Naghavi M, Mokdad A, Begum N, Shah R, Karyana M, Kosen S, Farje MR, Moncada G, Dutta A, Sazawal S, Dyer A *et al.* (2012) Common values in assessing health outcomes from disease and injury: disability weights measurement study for the Global Burden of Disease Study 2010. *Lancet* **380**, 2129-2143.
- Schmidlin T, Hürlimann E, Silué KD, Yapi RB, Hounbedji C, Kouadio BA, Acka-Douabélé CA, Kouassi D, Ouattara M, Zouzou F, Bonfoh B, N'Goran EK, Utzinger J, Raso G (2013) Effects of hygiene and defecation behavior on helminths and intestinal protozoa infections in Taabo, Cote d'Ivoire. *PLoS One* **8**, e65722.
- Spiegel JM, Dharamsi S, Wasan KM, Yassi A, Singer B, Hotez PJ, Hanson C, Bundy DA (2010) Which new approaches to tackling neglected tropical diseases show promise? *PLoS Med* **7**, e1000255.
- Steinmann P (2013) Effectiveness of latrines and chemotherapy for the control of soil-transmitted helminths in Yunnan, China (Personal communication).
- Steinmann P, Du ZW, Wang LB, Wang XZ, Jiang JY, Li LH, Marti H, Zhou XN, Utzinger J (2008) Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. *Am J Trop Med Hyg* **78**, 760-769.
- Steinmann P, Utzinger J, Du ZW, Jiang JY, Chen JX, Hattendorf J, Zhou H, Zhou XN (2011) Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp.: a randomized controlled trial. *PLoS One* **6**, e25003.
- Steinmann P, Zhou XN, Du ZW, Jiang JY, Wang LB, Wang XZ, Li LH, Marti H, Utzinger J (2007) Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and comparison of diagnostic methods. *PLoS Negl Trop Dis* **1**, e75.
- Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A (1993) Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr* **123**, 1036-1046.
- Stephenson LS, Latham MC, Kinoti SN, Kurz KM, Brigham H (1990) Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg* **84**, 277-282.

- Stephenson LS, Latham MC, Ottesen EA (2000) Malnutrition and parasitic helminth infections. *Parasitology* **121 Suppl**, S23-38.
- Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P (2012) Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin and school performance. *Cochrane Database Syst Rev* **11**, CD000371.
- TopEndSports (2013) Standing broad jump test (<http://www.topendsports.com/testing/tests/longjump.htm>; accessed August 2013).
- Utzinger J, Raso G, Brooker S, De Savigny D, Tanner M, Ornberg N, Singer BH, N'Goran E K (2009) Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. *Parasitology* **136**, 1859-1874.
- Utzinger J, Zhou XN, Chen MG, Bergquist R (2005) Conquering schistosomiasis in China: the long march. *Acta Trop* **96**, 69-96.
- Vedul-Kjelsås V, Sigmundsson H, Stensdotter AK, Haga M (2012) The relationship between motor competence, physical fitness and self-perception in children. *Child Care Health Dev* **38**, 394-402.
- Vercruysse J, Behnke JM, Albonico M, Ame SM, Angebault C, Bethony JM, Engels D, Guillard B, Nguyen TV, Kang G, Kattula D, Kotze AC, McCarthy JS, Mekonnen Z, Montresor A *et al.* (2011) Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. *PLoS Negl Trop Dis* **5**, e948.
- Wang L, Utzinger J, Zhou XN (2008) Schistosomiasis control: experiences and lessons from China. *Lancet* **372**, 1793-1795.
- WHO (2006) Preventive chemotherapy in human helminthiasis: coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme managers. Geneva, World Health Organization.
- WHO (2012) Eliminating soil-transmitted helminthiasis as a public health problem in children. Geneva, World Health Organization.
- Xiao S, Tanner M, N'Goran EK, Utzinger J, Chollet J, Bergquist R, Chen M, Zheng J (2002) Recent investigations of artemether, a novel agent for the prevention of Schistosomiasis *japonica*, *mansoni* and *haematobia*. *Acta Trop* **82**, 175-181.
- Yap P, Du ZW, Chen R, Zhang LP, Wu FW, Wang J, Wang XZ, Zhou H, Zhou XN, Utzinger J, Steinmann P (2012) Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. *Parasit Vectors* **5**, 50.
- Yap P, Du ZW, Wu FW, Jiang JY, Chen R, Zhou XN, Hattendorf J, Utzinger J, Steinmann P (2013a) Rapid re-infection with soil-transmitted helminths after triple-

dose albendazole treatment of school-aged children in Yunnan, People's Republic of China. *Am J Trop Med Hyg* **89**, 23-31.

Yap P, Utzinger J, Hattendorf J, Steinmann P (2013b) Influence of nutrition on infection and re-infection with soil-transmitted helminths: a systematic review. *Trop Med Int Health* **Submitted**.

Yap P, Wu FW, Du ZW, Hattendorf J, Chen R, Jiang JY, Kriemler S, Krauth SJ, Zhou XN, Utzinger J, Steinmann P (2013c) Effect of deworming on physical fitness of school-aged children in Yunnan, China: a double-blind, randomized, placebo-controlled trial. *PLoS Negl Trop Dis* **Under review**.

Yuan H, Jiagang G, Bergquist R, Tanner M, Xianyi C, Huanzeng W (2000) The 1992-1999 World Bank Schistosomiasis Research Initiative in China: outcome and perspectives. *Parasitol Int* **49**, 195-207.

Zhou H, Watanabe C, Ohtsuka R (2007) Impacts of dietary intake and helminth infection on diversity in growth among schoolchildren in rural south China: a four-year longitudinal study. *Am J Hum Biol* **19**, 96-106.

Zhou XN, Wang LY, Chen MG, Wu XH, Jiang QW, Chen XY, Zheng J, Utzinger J (2005) The public health significance and control of schistosomiasis in China - then and now. *Acta Trop* **96**, 97-105.

Ziegelbauer K, Steinmann P, Zhou H, Du ZW, Jiang JY, Furst T, Jia TW, Zhou XN, Utzinger J (2010) Self-rated quality of life and school performance in relation to helminth infections: case study from Yunnan, People's Republic of China. *Parasit Vectors* **3**, 61.

14. Curriculum Vitae

PERSONAL PARTICULARS

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EDUCATION

September 2010-Present Swiss Tropical and Public Health Institute (Switzerland)

- Ph.D., Epidemiology (Expected date to finish: October 2013)
- Ph.D. Thesis: Epidemiology and burden of soil-transmitted helminth infections among school-aged Bulang children in Yunnan province, People's Republic of China
- Supervised by Dr Peter Steinmann and Prof Dr Jürg Utzinger

2007-2009 National University of Singapore (NUS) and University of Basel (Switzerland)

- Master of Science (Joint MSc Program in Infectious Diseases, Vaccinology and Drug Discovery)
- M.Sc. Thesis: I. Mechanistic studies of anti-malarial spiroindolones and II. Synthesis and structure-activity relationship studies of an inhibitor of dengue proliferation
- Supervised by Dr Thomas Keller (Novartis Institute of Tropical Diseases, NITD) and Dr Sebastian Sonntag (NITD)

2002-2007 National University of Singapore

- Bachelor of Science with 2nd class upper honours (Pharmacy)
- Minor in Technopreneurship
- CAP : 4.19 (out of 5)

2005-2006 University of Pennsylvania (United States of America)

- NUS Overseas College Entrepreneurship Program (Bio Valley)
- Completed courses in entrepreneurship and product design

2000-2001 Hwa Chong Junior College (Singapore)

- Emphasis in Physics, Chemistry and Biology

WORK EXPERIENCE

September 2010-Present Swiss Tropical and Public Health Institute (Switzerland)

PhD Candidate

- Established a new topic of research on soil-transmitted helminths, physical fitness and anthropometric indicators among school children in rural China through collaboration with local researchers and authorities
- Conducted cross-sectional surveys and randomized controlled trials in resource-poor settings
- Attended Swissmedic accredited courses on Good Clinical Practice (GCP)
- Analyzed large data sets with STATA
- Drafted research proposals for funding applications, prepared ethical clearance applications and published peer-reviewed scientific journal articles

June 2011-December 2012 Swiss Center for International Health (Switzerland)

Part-time Assistant

- Edited reports for the Global Fund
- Analyzed and reported data from a national survey on intestinal helminths in Tajikistan

September 2009-August 2010 ETH Zurich (Switzerland)

Research Assistant

- Contributed towards the synthesis of cyclomarin analogs
- Tested conditions for solid-phase peptide synthesis

May-June 2007 Novartis Institute for Tropical Diseases (Singapore)

Research Assistant (Intern)

- Carried out solid-phase peptide synthesis for a dengue project
- Attended lectures and seminar on dengue and organic synthesis

July 2005-June 2006 RheoGene Inc. (United States of America)

Research Assistant (Intern)

- Performed formulation studies and organic synthesis of RheoGene proprietary chemical compounds
- Worked on a business case study with regards to social entrepreneurship and RheoGene's role in an insecticide against malaria project

Dec 2004 Institute of Bioengineering and Nanotechnology (Singapore)

Research Assistant (Intern)

- Fabricated drug loaded micelles and performed particle size analysis, loading level determination and encapsulation efficiency tests on them
- Cultured human breast cancer cells and carried out in vitro drug release studies of micelles on them

May 2004 Guardian Pharmacy (Causeway Point Branch, Singapore)

Pharmacy Assistant (Intern)

- Counseled patients and dispensed medicines (pharmacy only and prescription drugs) under preceptor's supervision
- Performed inventory management and retail marketing

June-September 2003 Undergraduate Research Opportunities Programme (Singapore)

- Independently studied the effects of grapefruit juice, orange juice and pummelo juice on hepatic and intestinal P-glycoprotein expression in mice
- Worked on Western Blot, animal models and protein preparations
- Grade obtained: A

AWARDS AND FUNDING

2012	Reisefonds University of Basel (travel grant of CHF 1'446.-)
2012	National Natural Science Foundation of China (project grant of RMB 100,000.-; together with Dr Peter Steinmann) 2012
2011	Freiwillige Akademische Gesellschaft, Basel, Switzerland (project grant of CHF 12'000.-; together with Dr Peter Steinmann)
2007-2009	Scholarship from NITD for the Joint MSc Program
2004-2005	Dean's List (NUS)
2000-2001	ASEAN Pre-University Scholarship

PUBLICATIONS

Peer-reviewed articles:

1. **Yap P**, Du ZW, Chen R, Zhang LP, Wu FW, Wang J, Wang XZ, Zhou H, Zhou XN, Utzinger J, Steinmann P, 2012. Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. *Parasites & Vectors* 5(1):50
2. **Yap P**, Fürst T, Müller I, Kriemler S, Utzinger J, Steinmann P, 2012. Determining soil-

- transmitted helminth infection and physical fitness of school-aged children. *Journal of Visualized Experiments* (66): e3966
3. Zou B, **Yap P**, Sonntag LS, Leong SY, Yeung B, Keller T, 2012. Mechanistic study of the spiroindolones: a new class of anti-malarials. *Molecules* 17(9): 10131 – 10141
 4. Qian MB*, **Yap P***, Yang YC, Liang H, Jiang ZH, Li W, Tan YG, Zhou H, Utzinger J, Zhou XN, Keiser J, 2013. Efficacy and safety of tribendimidine against *Clonorchis sinensis*: a randomized, exploratory, open-label trial. *Clinical Infectious Diseases* 56(7): e76 – 82
 5. Sherkhonov T*, **Yap P***, Mammadov S, Amoss WP, Wientzen RL, Steinmann P, 2013. National intestinal helminth survey among schoolchildren in Tajikistan: prevalences, risk factors and perceptions. *Acta Tropica* 126(2): 93 – 98
 6. **Yap P**, Du ZW, Wu FW, Jiang JY, Chen R, Zhou XN, Hattendorf J, Utzinger J, Steinmann P, 2013. Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in Yunnan, People's Republic of China. *American Journal of Tropical Medicine and Hygiene* 89(1); 23 – 31
 7. **Yap P**, Utzinger J, Steinmann P, 2013. Impact of undernutrition on infection and re-infection with soil transmitted helminths: a systematic review. *Tropical Medicine & International Health* Submitted
 8. Qian MB, **Yap P**, Yang YC, Liang H, Jiang ZH, Li W, Utzinger J, Zhou XN, Keiser J, 2013. Accuracy of the Kato-Katz and ether-concentration methods for the diagnosis of *Clonorchis sinensis* and effect on assessing drug efficacy. *Parasites & Vectors* Under review
 9. **Yap P**, Wu FW, Du ZW, Chen R, Jiang JY, Wang J, Hattendorf J, Kriemler S, Krauth SJ, Zhou XN, Utzinger J, Steinmann P, 2013. Effect of de-worming on the physical fitness of children in Yunnan, China: a double-blind, randomized, placebo-controlled trial. *PLoS Neg Trop Dis* Submitted

*Co-first authors

Book chapter:

1. Utzinger J, **Yap P**, Lv S, Yang GJ, Zhou XN, Steinmann P, Molyneux D, 2013. Chronic neglected diseases of poverty in Asia: challenges and opportunities ahead. *Routledge Handbook of Public Health in Asia: a Global Perspective* In press

Peer-reviewed for the following journals:

- Infectious Diseases of Poverty
- Acta Tropica
- Parasites & Vectors
- PLoS Neglected Tropical Diseases
- The Lancet Global Health

CONFERENCES

10-13 September 2013 8th European Congress of Tropical Medicine and International Health (Copenhagen)

- Presented a talk “Effectiveness of latrines and chemotherapy for the control of soil-transmitted helminths in Yunnan, China” (Session: Sanitation in the post-2015 landscape)

11-15 November 2012 61st Annual meeting of the American Society of Tropical Medicine and Hygiene (United States of America)

- Attended the global health pre-meeting course entitled “Building global public health and research capacity: a discussion of three case studies and lessons learned”
- Presented a poster “Soil-transmitted helminths and physical fitness among Bulang children in Yunnan, China” (Poster No.: 122)
- Presented a late breaker poster “Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment” (Poster No.: LB 202)

VOLUNTEER/COMMUNITY WORK

April–May 2007 Visualising Issues in Pharmacy Project (Singapore)

Pharmacy Participant

- Conducted literature research on how to increase public awareness of malaria in Winam, Kenya
- Composed (team effort) a report on issues of malaria to be included in visual campaigns for the reference for design students

June 2004-June 2005 Fei Yue Volunteer Center (Singapore)

Volunteer

- Volunteered in camps for juveniles under Project 180

June 2002 Youth Expedition Project to Hlaing Thayar (Myanmar)

Participant/ First Aider

- Participated in the building of a clinic in a village in Myanmar
- Played the role of a certified First Aider on the expedition

LANGUAGE AND COMPUTER SKILLS

English: Advanced (read, speak, write)

Mandarin: Native

Malay: Basic (speak)

German: Basic (read, speak, write)

Computer Skills

MS Office

Stata

INTERNATIONAL EXPERIENCES

Lived and worked in: Malaysia, Singapore, China, Switzerland and the United States of America



Peiling Yap

1.10.2013